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# INDUSTRIAL FERMENTATIONS

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# PREFACE

Certain fermentations are among the oldest chemical processes which have been conducted by mankind. These ancient fermentations were operated for the production of alcoholic beverages, for vinegar production, for leavening of bread and for the preparation of food products.

Many industrial fermentations of today had their origin in some of these old processes. With the rise of organic chemistry, the demand for organic chemicals led to the adaption on a large scale of some of the fermentation procedures for the manufacture of certain of these chemicals. However, it is only since the beginning of this century that fermentation processes have been put on a scientific basis. At the same time, new fermentations have also been developed specifically for the production of additional chemical substances. During this period, commercial fermentations have expanded enormously and their scope has widened to such an extent that today few industrial enterprises are not affected in some way, either directly or indirectly, by fermentation processes.

Because of the rapid advances in the fermentation industries and also because of somewhat meager publication by industrial concerns, it is the opinion of the editors that no one or two indivi-

duals could authoritatively cover the entire field of industrial fermentations. Therefore, these volumes have taken the form of a symposium on many of the phases of industrial fermentations. Most chapters are written by contributors who have had considerable experience with their selected topics. Many of the chapters are written by individuals who are associated with industries which have made a financial success of the fermentations described. Other chapters are contributed by research workers with an academic background who specialize in developing new fermentative processes.

Most of the chapters are limited primarily to industrial processes which are or have been commercially important. However, a few topics are included which, while not at present industrially utilized, are potentially important; that is, they have been or are being considered for commercial exploitation. Such processes include, for example, those for the preparation of 2, 3-butanediol and some of the ketogenic processes employing *Acetobacter* or *Pseudomonas* organisms.

Some topics which might be technically considered as commercial fermentations in the broad sense are not included. These are generally microbiological processes of specialized nature, such as the panary fermentation, retting, ensiling, legume inoculation, and other such procedures, which are adequately described in other publications. With few exceptions, the topics included are largely restricted to the commercial fermentative production of specific chemicals.

The editors have not been unaware of the many pitfalls to be encountered in presenting a subject of such ramifications. Variations in style and detail of treatment in the different chapters will be apparent to the reader. The editors are also regretfully aware that some of the information given will be out-dated before these pages appear in print. For example, in such rapidly expanding fields as the production of antibiotics by fermentation, the printed page cannot hope to keep up with the newest developments. While every effort has been made by the authors and editors to avoid errors of omission and commission, there are undoubtedly some of each kind in this book. For any such errors the editors bespeak the indulgence of readers and offer in advance their apologies to the investigators who are the victims of these errors.

Despite imperfections and omissions, we believe that these

volumes are unique in the authoritative presentation of one of the most important branches of chemical industry. The rapid advances and current importance of the fermentation industries should make such a compilation of the current knowledge and industrial practice of much value both to those in the industry and to students interested in fermentations. It is hoped that these volumes will prove to be stimulating, enlightening, and educational to those in industry as well as to students in colleges and universities.

The editors wish again to express their deep appreciation to the contributors who have been most generous with their time and effort in this cooperative undertaking. Each writer, of course, made his contribution as an individual, and not as a representative of the institution or organization with which he is associated. A word of appreciation is also due from the editors and from the chapter authors to those individuals, who must go unnamed because they are so numerous, who made available bibliographies and unpublished information, and who read chapters or sections of chapters and were so generous with their helpful criticisms.

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# INTRODUCTION

*L. A. Underkofler and R. J. Hickey*

The fermentation industries constitute the branch of chemical manufacture which yield useful products through the vital activities of microorganisms. *Fermentation,* in the broad sense in which the term is now generally used, may be defined as a metabolic process in which chemical changes are brought about in an organic substrate through the activities of enzymes secreted by microorganisms.

The term "fermentation" underwent numerous changes in meaning during the past century. It is derived from the Latin *fervere* meaning "to be boiling," and originally signified the gentle bubbling or boiling condition observed in the spontaneous transformation of fruit juices, as in wine or cider fermentation. The meaning was changed through Gay-Lussac's study of alcoholic fermentations to indicate the conversion of sugar into carbon dioxide and alcohol. Pasteur's researches as to the cause of fermentative transformation led him to define fermentation as "life without air." Now the word has become more closely associated with the microorganisms and the enzymes secreted by them which catalyze the fermentative changes.

With respect to oxygen supply, two types of fermentation are

recognized. *Aerobic fermentation* (oxybionic processes; respiration) is the form of dissimilation which requires free oxygen to act as a hydrogen acceptor. The acetic acid and citric acid fermentations are examples. *Anaerobic fermentation* is dissimilation in which atmospheric oxygen is not involved, but other substances, such as aldehydes or pyruvic acid, serve as hydrogen acceptors. Examples are the alcoholic, butyl alcohol-acetone, and lactic acid fermentations.

Literally thousands, if not millions, of different kinds of microorganisms exist in nature, and each of these produces fermentative changes in the broad sense. These microbial dissimilations are of the most diverse kinds, but the number of transformations which have become of technical importance is quite limited. Success of a fermentation process on the industrial scale depends on a number of factors, notably: (1) the ability of the selected organism to give a consistently high yield of the desired product in a reasonable time from a cheap, available raw substrate; (2) the easy recovery of the product in pure form; and (3) the manufacture of a unique product which is in demand, but difficult to obtain by other methods.

Organisms of various types are employed in the fermentation industries. These include species or strains of yeasts, bacteria, and molds. The microorganisms of fermentation differ greatly in morphology, size, manner of reproduction, reaction to free oxygen, growth requirements, ability to attack different substrates, and in other ways. They are similar in that they grow actively and produce enzymes by which they catalyze the reactions brought about by them.

It is now well known by microbiologists and biochemists that enzymes are biochemical catalysts produced by living cells. In general, their action is independent of the living cell; they are thermolabile and frequently very specific in their catalytic actions. The various enzymes of living cells, under the mildest conditions, bring about a vast number of complicated reactions, many of which are still beyond the understanding of the organic chemist. The enzymes are of two types. *Exoenzymes* are excreted by the cells and act outside the cell on organic substances, such as proteins, carbohydrates, and fats as substrates, forming derivatives which are generally soluble and can be absorbed through the cell membrane. *Endoenzymes* are elaborated and retained within the living cell and catalyze the transformation or dissimilation of food substances within the cell. The products of this dissimilation may be built

up into cell protoplasm, or excreted through the cell membrane. The chemical changes resulting from the enzyme actions cause the liberation of relatively large amounts of energy available to the cell for its metabolic activities.

The microorganisms of fermentation are notable for their ability to produce enzymes of both classes. Sometimes microorganisms are employed as industrial sources of exoenzymes, such as amylases and proteases. More commonly, in industrial fermentation processes, the products of chemical action excreted from the cells as a result of endoenzyme action are isolated for practical uses.

The industrially important microorganisms have at least five outstanding abilities:

(1) To grow rapidly in suitable organic substrates;

(2) To be cultivated easily in large quantities;

(3) To produce the necessary enzymes readily and profusely, in order to bring about the desired chemical changes;

(4) To carry out the transformations under comparatively simple and workable modifications of environmental conditions;

(5) To maintain physiological constancy under these conditions.

Industrial operations with microorganisms are obviously more complicated than laboratory work, but involve the same principles. The chemical engineering equipment must be carefully designed to meet the special conditions required in each type of fermentation and must include means to protect against contamination by other organisms.

The best-known chemical product obtained industrially by fermentation, and the one of largest volume, is ethyl alcohol, but other fermentation chemicals, including *n*-butyl alcohol, acetone, lactic acid, citric acid, sorbose, gluconic acid, itaconic acid, glycerol, 2,3-butanediol, riboflavin and other vitamins, penicillin and other antibiotics, various enzymes, and other substances, are or have been also produced on commercial or pilot-plant scale. Still other processes for producing chemicals by fermentation are continually under investigation in laboratory and pilot plant. Some of these may lead to important new fermentation industries in the future.

Fermentations are also of great economic importance in industries not directly concerned with the production of chemical substances as such. For example, manufacture of certain beverages, bakery products, dairy products, sauerkraut, pickles and other foods,

frequently involve microbiological procedures. Microbial action is of great importance in sewage disposal plants. Microbiological-assay procedures for vitamins and other nutrient factors are in common use because of their relative simplicity, specificity, and rapidity. The commercial production of sera, vaccines, and other therapeutic agents, besides antibiotics, constitutes an important branch of industrial microbiology. Likewise, soil microbiology, the use of nitrogen-fixing bacteria, and the study of the phytopathology of economic crop plants are of the greatest importance in agriculture and thus of industrial and economic significance. However, fermentations, in many cases, are undesirable. Food spoilage by fermentation results in economic loss. Disease organisms produce illness or even death in plant, animal, or human hosts. In general, such processes as mentioned in this paragraph, whether they are useful or undesirable, are beyond the scope of this book.

The discussions in this volume will be limited to industrial fermentation processes in which chemical compounds are isolated for use in chemical and other industries. Particular emphasis will be placed on the art of controlling and investigating technical fermentation processes, that is, on using microorganisms as catalytic tools to produce desirable end products having well-defined industrial applications. Those processes which are at present of industrial importance will be given chief attention, with some consideration also of other processes which have in the past been utilized on the commercial scale, or have reasonable prospects for future industrial exploitation. Although all the minute details of each industry cannot be given in a book of this scope, an attempt is made to present the principles and the most up-to-date general methods of technical operation and control.

Chemical manufacture by fermentation is characterized by a high degree of flexibility as to raw materials. In almost all cases, the raw materials for fermentation processes are directly or indirectly the products of agriculture and the fermentation industries are most commonly cited as examples of "chemurgy," which has come to mean the production of useful chemicals from farm crops. Since farm products are the primary raw materials for commercial fermentations, the wide fluctuations in availability and prices of farm crops have at times been a serious handicap to the industries. However, in many processes, a wide variety of raw materials may be employed for a given fermentation, and thus advantage may be

taken of availability and price changes. One example was the shift from grain to molasses for producing fermentation butanol and acetone before World War II, because of greater price stability and lower cost for molasses as compared with grain. Another example was the shift from molasses to grain for industrial alcohol production during the same war, when lack of shipping facilities and available quantities of molasses prevented an adequate supply of molasses for meeting the war demands for ethanol.

The majority of large-scale fermentation processes involves carbohydrate dissimilation. In general, two classes of carbohydrate raw materials for fermentation processes are available: (1) saccharine substances and (2) polysaccharides. It should be noted, however, that while most of the major fermentations of industrial importance involve transformations of carbohydrates, in some cases, other substances are the essential substrates, for example, sugar alcohols, proteins, or lipids.

In some processes, the saccharine substance used for an industrial fermentation is pure sugar, such as sucrose, glucose, or lactose. These are used where special conditions of process, product demand, or other factors make the higher cost of pure sugar permissible. In most cases, the saccharine substances employed are cheap by-products, and have other ingredients along with the fermentable sugars.

Blackstrap molasses, a by-product from the manufacture of cane sugar, has been generally the lowest-cost raw material for the fermentation industries. Blackstrap molasses contains about 50% fermentable sugars, mainly sucrose and invert sugar, and also inorganic compounds, nitrogenous substances, and other organic compounds of diverse nature. Beet molasses is a similar by-product from the manufacture of beet sugar. In times of surplus production of sugar cane, high-test or invert molasses is available. This is produced by inverting the sugar in whole cane juice by heating at acid reaction, neutralizing, and evaporating to about 75% sugar content. According to Gabriel,[9] 339 million gallons of high-test molasses were imported into the United States in 1941, with an importation of 101 million gallons of blackstrap molasses in the same year.

Other saccharine by-products which have been used or suggested for fermentation are whey, sulfite waste liquor from the paper-pulp industry, corn molasses or hydrol, cull fruits and cannery wastes of various kinds, citrus-fruit juices, pineapple juice, etc.

Polysaccharide raw materials which are used for fermentation processes usually contain starch. Pure starch has been proposed for certain fermentations and large-scale use of grains, including corn, wheat, rye, milo, and rice, has been practiced frequently in many countries. Potatoes and sweet potatoes are other available starchy raw materials and in tropical regions, cassava offers much promise.

The manufacture of alcohols and other chemicals from grain starches reached a high level in the United States during World War II. Boruff and Van Lanen[4] have summarized the production data. The consumption of grains for ethanol fermentation as reported by Jacobs[12] totaled about 420 million bushels for the 4 war years 1942-1945. Since the war, high prices for grains have restricted their use in fermentation industries. However, potatoes, which have been produced in large surplus, have been employed in considerable quantities.

Other polysaccharide raw materials which have been proposed for fermentation use are those containing inulin, such as the Jerusalem artichoke, and those containing cellulose, which is the most abundant organic compound produced in nature. Wood waste from the lumbering industry contains about 50% cellulose. Agricultural wastes or residues, such as oat hulls, corn cobs, cottonseed hulls, peanut shells, corn stalks, straw, etc., contain a similar proportion of cellulose. Utilization of cellulosic wastes for fermentation processes necessitates preliminary saccharification of the cellulose. Since cellulose is resistant to hydrolysis, drastic treatment is necessary, which has proved to be somewhat costly. Nevertheless, in European countries, where the production of agricultural crops containing sugars and starch is inadequate for food purposes, the saccharification of wood waste for the fermentation industries has become an established commercial practice. In the United States, toward the end of World War II, a plant was built in the Pacific Northwest to produce alcohol by saccharification and fermentation of sawmill wastes. This plant was operated for only a very short period. A process for converting agricultural residues, such as corn cobs, has been developed for the production of sugar solutions, as separate pentose and hexose fractions, for possible use in the fermentation industries.[7]

Since the fermentation industries, in general, employ as raw materials the products of agriculture: primary products, such as grains; derived products, such as sugars, starches, or high-test molas-

ses; and by-products, such as blackstrap or beet molasses, agriculturists and industrialists have become interested in fermentation proccesses. Chemurgists seek to promote the use of agricultural products in industry, especially as a buffer against overproduction of farm crops in the United States. Before World War II, it was commonly accepted that American agriculture had a productive capacity for grain crops about 12% above domestic and foreign market requirements. During the war, through technological advances, agricultural productivity expanded about 30 to 35% in this country, according to government estimates.[1] Although surpluses in most crops have not been experienced since the war, largely due to export of farm crops, it is possible that agricultural surpluses will again appear in this country. If there will be surpluses, they are quite likely to be considerably above those of the prewar period and may be particularly large for grains. But surpluses of grains are not true surpluses of all grain constituents. The analyses by Shepherd, McPherson, Brown and Hixon,[18] Filley, Loeffel and Christensen,[8] Christensen,[5] and others have shown that the important ingredients, except starch, of the so-called grain surpluses are needed in the national economy. This is particularly true of proteins and fats which have always been imported to meet requirements. The problem, then, is one of finding uses for the starch. The use in fermentation processes is, of course, of great interest in this connection and is the subject of several recent analyses. Those of Groggins,[9] Filley, Loeffel and Christensen,[8] Jacobs,[12] and Hilbert[11] are good indications of current thinking in agricultural groups.

A real appraisal of the economic position of the different available raw materials for fermentation industries is indeed difficult. Most of the appraisals which have been attempted have covered only a single fermentation industry. Many of these appraisals are now rather old[14,18] and others are incomplete.[3,13,20] A brief, but very general, comparison of the possible competitive positions of the major raw materials is given in the following paragraphs.

Obviously, by-product materials, such as molasses, have the lowest value and will sell at almost any figure below competitive costs of other raw materials available for equivalent uses. Gabriel[9] has indicated that if there were no sale value, cane-sugar mills could better afford to give away their molasses than to go to the expense

of disposing of it. Certainly blackstrap molasses will continue to be a favored raw material for fermentation industries. Likewise, in periods of sugar-cane production in excess of the demand for sucrose, high-test molasses will be an important factor in the economic position of fermentation raw materials. An increasing tendency to utilize molasses for fermentation in the countries where it is produced may decrease its availibility in other countries which import molasses. The utilization of molasses in the countries of origin is a healthy condition, but is limited by the demand for the fermentation products and by the availability of processing water and other facilities. Aries[3] and Gabriel[9] have reviewed the molasses situation and attempted to predict future trends.

Another saccharine by-product which is achieving increased utilization as a fermentation substrate is the waste liquor from sulfite-pulp mills. This residue had no economic value in the past and, in many cases, constituted a serious disposal problem, being a distinct nuisance as regards stream pollution. Scandinavian countries have been using sulfite waste liquor for a long time as a fermentation substrate. More recently, plants for production of ethanol and yeast from sulfite waste liquor have gone into operation both in Canada and the United States. Tousley[20] has indicated that sulfite waste liquor is one of the cheapest raw materials for the fermentative production of ethanol and yeast and that alcohol from waste sulfite liquor should be able to compete with alcohol from molasses. The potential production of ethanol from this source, should all the waste liquor available in the United States be utilized for this purpose, would probably be somewhat in excess of 50 million gallons per year.[20] However, plants producing alcohol from this source must be constructed and operated in conjunction with sulfite-pulp mills and, in many cases, limitations in the size of the mill, availability of supplies and water, location, and other factors would not warrant the operation of alcohol plants by all sulfite-pulp mills. Tousley[20] estimates the practicable maximum output of alcohol from waste sulfite liquor at 25 million gallons. Increasing use of this by-product for ethanol and yeast manufacture may be expected, both as a means of securing a monetary return from an otherwise wasted by-product and as a means of combating the disposal nuisance of sulfite-pulp plants.

Whey, or lactose from whey, is a potential large-scale source of fermentation chemicals. Smith and Claborn[19] have estimated that a

total of 2.7 billion pounds of lactose could be obtained from skim milk and other dairy by-products, a large percentage of which could be made available for fermentation. Whey contains about 5% lactose. Since milk and, therefore, also whey contain large numbers of microorganisms, spontaneous fermentation takes place rapidly. For application in fermentation industries, whey would have to be used at the source or lactose separated from it for subsequent use. Fermentation industries producing antibiotics and lactic acid now utilize this source of carbohydrate to a certain extent. Lactose is currently employed in large amounts in the penicillin fermentation.

Raw materials containing starch, *e.g.*, the grains and potatoes, are raised primarily for food or feed purposes. The food and feed markets for grains command higher prices than most fermentation industries can afford. For profitable utilization of grains by fermentation industries, special factors must be operative. Since primarily only the starch of the grains is fermented, in some cases, by-product credits for other grain components may make fermentative utilization feasible. For example, from wheat, the gluten may be isolated and used for the manufacture of monosodium glutamate. The residual starch then serves as an economical source of fermentation carbohydrate. From corn, the corn oil and protein feed concentrates or steep liquors are by-products which lower the cost of the starch for fermentative utilization. It has been suggested that by suitable extraction procedures, a high-quality wax can be obtained from sorghum grains (milo), which, along with oil and protein feed credits, may have sufficient monetary value to allow the use of residual starch for fermentation.

Suggestions for the application of grain surpluses by fermentation industries have been numerous. It was also proposed to subsidize fermentation industries or farmers in various ways so that grain surpluses could be economically used in fermentation processes. Since of the present-day fermentation processes only the alcoholic fermentation industry is sufficiently large to absorb appreciable quantities of grain surpluses, greatest emphasis has been placed on this fermentation as an outlet for surpluses.

Although markets for industrial ethanol have been expanding, present uses would not absorb the large amounts of alcohol which could be produced from possible grain surpluses. Very large new outlets for alcohol would be required for this purpose. A familiar proposal has been to utilize excess grains for production of alcohol

for blending with gasoline as motor fuel for internal-combustion engines. Although technically feasible, this proposal has met great opposition in the United States on the grounds that it is not economic to blend the relatively higher priced alcohol with cheaper gasoline. Many foreign countries, where petroleum must be imported, have quite generally employed alcohol blends and, during 1936-1937, more than 15 million gallons of alcohol-blend fuels were distributed in the middlewest of the United States[18] with entire satisfaction to the users of this fuel in motor cars.

Recently, considerable interest[6,10,15,16,17] has developed in the possibility of employing alcohol as a supplemental fuel for internal-combustion engines. In this system, an alcohol-water mixture is carried in a separate tank and is admitted to the intake manifold by means of an automatically controlled metering device that injects the alcohol when conditions are favorable for detonation, i.e., open throttle and relatively low piston speed in most engines. This system would be well adapted to use with very-high-compression engines. Theory indicates, and apparently tests confirm, that by using compression ratios of 10:1 and higher, fuel consumption by automotive equipment might be reduced by as much as 50%. Several large automobile manufacturing concerns are apparently ready to produce such high-compression engines for stock automobiles, but the available fuels limit the usable compression ratio to about 7.5:1. Very high octane rating fuels are required for the high-compression engines, but this requirement is only at periods of full throttle and high load. Automobiles equipped with high-compression engines should operate very efficiently with a dual fuel system, using conventional motor fuels and alcohol injection to prevent detonation and promote full efficiency under peak load and open throttle. It should be noted that methanol and isopropyl alcohol can be used as well as ethanol in this manner and all three have substantially the same value for this purpose. Should this system be generally adopted, with all automotive equipment in the United States so equipped, the consumption of alcohols would reach about a billion gallons per year for this service. This would be a tremendous outlet for fermentation ethanol and synthetic alcohols, which might well take care of all foreseeable grain surpluses and even require the growing of crops primarily for alcohol production.

The economic position of wood waste and agricultural residues as potential raw materials for fermentation seems to be less

subject to speculation and controversy than that of other available substances. They will undoubtedly continue to be employed and their utilization be expanded in countries which cannot raise sufficient food crops to meet the requirements of their population. However, the drastic conditions necessary for the saccharification of cellulosic materials result in high plant costs and high processing costs, making these materials uneconomic in the United States in comparison with other available fermentation substrates. Recent reports indicate that, under certain favorable conditions of location, utilization of agricultural residues by a newly developed process,[2,7] might be feasible industrially and research on the utilization of all sorts of cellulosic materials for fermentation may be expected to continue.

A consideration of raw materials for fermentation industries would not be complete without mention of the importance of adequate water supplies. While water is not directly a substrate for fermentation, microorganisms act in aqueous media and any fermentation industry is basically dependent on the availability of very large supplies of water. This water is required for preparation of fermentation mashes, for steam production, for processing operations, and especially for cooling. Fermentation plants have failed just because of inadequate supplies of cold water! The design data for the industrial alcohol plant, built and operated for the United States government during World War II at Omaha, Nebraska, indicated a requirement of about 65 gallons of water per gallon of alcohol produced and during hot summer months, the actual consumption went as high as 80 gallons of water per gallon of alcohol. During the peak production periods, 4 to 7 million gallons of water were pumped at this plant daily. Probably the first major consideration in the location of a fermentation plant should be adequate supplies of good quality, cold water.

Fermentation industries are, in many cases, faced with severe competition by synthetic processes for the production of the same chemicals. For example, fermentation ethanol must compete with ethanol made from ethylene, a by-product of petroleum-cracking processes. Fermentation butanol and acetone also must compete with these compounds made synthetically from petroleum gases. There is considerable interest in the alcohols which are produced from operation of Fischer-Tropsch plants for production of synthetic fuels from natural gas or coal. Schroeder[9] has described the general

situation and has indicated that by this means, very-low-cost alcohols may become available in large amounts.

All branches of chemical industry must produce basic compounds at the lowest possible prices. The processes which survive are, therefore, those which are most economical. A fermentation industry will compete with synthetic processes only at utmost economy and efficiency. Some fermentation processes which are technically sound, such as isopropyl alcohol and glycerol production, have not been developed industrially because of the more favorable economic position of synthetic processes for these substances.

In some processes, however, the products of fermentation procedures are unique, i.e., these products have not been produced economically by synthetic methods. Citric acid, lactic acid, penicillin, and streptomycin, for example, at present have no serious competition from synthesis. Nevertheless, increased efficiencies and economies in each of these fermentation industries have resulted in markedly lowering the production costs and prices at which these chemicals are sold to the consumer.

In the following chapters, specific fermentation processes of actual or potential industrial significance are discussed in considerable detail. In connection with each fermentation, the historical or research background is summarized and the available raw materials are considered. The microbial cultures employed and the methods of their maintenance and cultivation are described. The procedures in laboratory and plant practice are discussed and, in many cases, are illustrated with flowsheets and equipment layouts. Consideration is given to by-products, their importance, and recovery. The economics of the process, use of the products, and competitive operations and processes are reviewed. Finally, minor variations and new developments are also discussed. In this manner, the book gives an over-all picture of the ramifications of Industrial Fermentations.

## BIBLIOGRAPHY

1. Anon., *Chem. & Met. Eng.*, **52**, No. 11, 114 (1945).
2. Anon., *Chem & Met. Eng.*, **53**, No. 12, 104 (1946).
3. Aries, R. S., *Chem. Eng. News*, **25**, 1792 (1947).
4. Boruff, C. S., and J. M. Van Lanen, *Ind. Eng. Chem.*, **39**, 934 (1947).

5. Christensen, L. M., p. 518 *in* R. W. Kerr, *Chemistry and Industry of Starch,* 2nd ed., New York, Academic Press, 1950.
6. Colwell, A. T., R. E. Cummings, and D. E. Anderson, *S. A. E. Journal* (Trans.), **53**, 358 (1945).
7. Dunning, J. W., and E. C. Lathrop, *Ind. Eng. Chem.,* **37**, 24 (1945).
8. Filley, H. C., W. J. Loeffel, and L. M. Christensen, *Nebr. Chemurgy Project Progress Report,* No. 9, Feb. 15, 1943.
9. Gabriel, C. L., L. M. Christensen, L. K. Burger, W. C. Schroeder, and P. H. Groggins, A Symposium, *Chem. Eng. Progress,* **44**, 13 (1948).
10. Heldt, P. M., *Automotive Inds.,* **98**, No. 2, 32 (1948).
11. Hilbert, G. E., *Chemurgic Digest,* **7**, No. 5, 8 (1948); Northern Regional Research Lab., Mimeo. OP-5499.
12. Jacobs, P. B., *Chemurgic Digest,* **7**, No. 5, 11 (1948); Northern Regional Research Lab., Mimeo. OP-5487.
13. Jacobs, P. B., *Industrial Alcohol,* Washington, D. C., U.S.D.A. Misc. Publ. No. 695, 1950.
14. Jacobs, P. B., and H. P. Newton, *Motor Fuels from Farm Products,* Washington, D. C., U.S.D.A. Misc. Publ. No. 327, 1938.
15. Mack, R. C., *Automotive Inds.,* **98**, No. 3, 36 (1948).
16. Porter, J. C., M. M. Gilbert, H. A. Lykins, and R. Wiebe, *Agr. Eng.,* **31**, No. 2, 71 (1950).
17. Porter, J. C., W. B. Roth, and R. Wiebe, *Automotive Inds.,* **98**, No, 8, 34 (1948).
18. Shepherd, G., W. K. McPherson, L. T. Brown, and R. M. Hixon, *Power Alcohol from Farm Products, Iowa Corn Research Institute,* **1**, No. 3, (1940).
19. Smith, L. T., and H. V. Claborn, *Ind. Eng. Chem., News Ed.,* **17**, 370 (1939).
20. Tousley, R. D., *The Economics of Industrial Alcohol,* State Coll. of Washington, Bureau Econ. and Business Research, Pullman, Wash., Bull. No. 3, August 1945; *Chem. & Met. Eng.,* **52**, No. 10, 120 (1945).

# Part I. ALCOHOLIC FERMENTATION AND ITS MODIFICATIONS

CHAPTER 2

# ALCOHOLIC FERMENTATION OF GRAIN

*W. H. Stark*

The alcoholic fermentation is the largest sector of the fermentation industries in terms of product quantity, number of commercial units, and persons employed directly and indirectly. The alcohol fermentation industry of today is an outgrowth of what may be the oldest chemical process carried out by mankind. The original use of alcoholic fermentation was, of course, for preserving fruit juices, and man's first volitional use of this fermentation is lost in the pages of antiquity. Later, the fermentation was adapted to the preparation of fermented grain beverages and then distilled beverages. In more modern times, production of alcoholic beverages became a sizable industry and governments found in alcohol a fertile field for the tax collector.

When synthetic organic chemistry began its rapid advance about a century ago, alcohol became an indispensable chemical, but its extensive manufacturing use was hampered by the excessive taxes. The problem was to devise a means for conserving governments' tax interest in potable alcohol and, at the same time, making alcohol available tax-free for industrial uses. The procedure evolved for accomplishing this end is technically known as "denaturation."

Most countries now permit the industrial use of denatured alcohol tax-free. Great Britain was the first country to authorize denaturation, in 1855 legalizing alcohol to which 10% of wood alcohol was added as "methylated spirits." It was not until the industrial alcohol act of 1906 that denatured alcohol was legalized in the United States. The manufacture and sale of alcohol is of necessity closely controlled by governmental agencies. In the United States, alcohol is produced in accordance with the regulations of the Bureau of Industrial Alcohol of the United States Department of the Treasury.[48]

During World War II, the demands for industrial ethyl alcohol were multiplied four- or fivefold above the prewar normal, due to its use in synthetic rubber and smokeless powder manufacture. With molasses imports inadequate at best and further curtailed by the enemy submarine campaign, the grain-alcohol process met the requirements. In peacetime, the economics of alcohol production are such that most of the industrial alcohol is derived by fermentation of molasses and by synthesis from petroleum sources, while most of the alcohol produced by fermentation of grains is used for beverages. Recently, Arnold and Kremer[4a] made an economic study of various processes for industrial alcohol production, placing particular emphasis on the future of corn as a raw material.

The data presented in Table 1 illustrate the size of the alcohol industry and the importance of the alcoholic fermentation of grains. Many plants operate under both industrial alcohol license and as registered distilleries so that the total number of physical units is somewhat smaller than the sum of the operating plants as shown. It is interesting to note the tremendous increase in alcohol production during the years of World War II and the high percentage of alcohol derived from grains during that period in both types of plants. In peacetime, large quantities of alcohol are produced from grains in registered distilleries and very little in industrial alcohol plants.

A breakdown of the raw materials used for ethyl alcohol production in the United States and its possessions is presented in Table 2. The principal raw materials used for the manufacture of fermentation alcohol in the United States are molasses and grains. However, other available substrates containing sugars or starch are potential raw materials for alcohol fermentation and are employed, to a certain extent, in this and other countries. The

TABLE 1. ALCOHOL PRODUCTION IN THE UNITED STATES AND THE QUANTITY OF GRAIN USED

| Year | *Industrial Alcohol Plants* | | | | *Registered Distilleries* | | | |
|---|---|---|---|---|---|---|---|---|
| | Operating plants | Grain used | Total proof | Derived from grain | Operating plants | Grain used | Total proof | Derived from grain[a] |
| | No. | lb $\times 10^3$ | gal | % | No. | lb. $\times 10^3$ | gal | % |
| 1939 | 36 | 180,447 | 201,017,446 | 7.73 | 112 | 1,327,113 | 145,326,176 | 80.0 |
| 1940 | 37 | 159,347 | 243,727,756 | 5.73 | 101 | 1,404,050 | 143,455,192 | 86.0 |
| 1941 | 39 | 199,120 | 298,845,417 | 5.87 | 105 | 1,713,549 | 175,208,746 | 86.0 |
| 1942 | 162 | 3,068,587 | 517,500,002 | 52.0 | Figures represent combined totals for both industrial alcohol plants and registered distilleries for these years. See footnote regarding percentage from grain. | | | |
| 1943 | 193 | 5,837,713 | 732,350,228 | 69.9 | | | | |
| 1944 | 188 | 7,268,283 | 987,957,702 | 64.6 | | | | |
| 1945 | 200 | 6,865,102 | 1,101,286,249 | 54.7 | | | | |
| 1946 | 48 | 1,254,452 | 442,417,716 | 29.69 | 144 | 3,075,505 | 305,066,637 | 88.5 |
| 1947 | 38 | 509,569 | 248,798,639 | 13.30 | 147 | 2,974,052 | 315,157,700 | 82.5 |
| 1948 | 47 | 421,465 | 332,282,148 | 9.44 | 130 | 2,491,507 | 244,127,343 | 89.5 |
| 1949 | 46 | 138,531 | 351,015,364 | 3.05 | 132 | 2,515,250 | 266,542,499 | 82.5 |
| 1950 | 38 | 27,562 | 313,535,129 | 0.74 | 119 | 2,061,899 | 208,235,050 | 91.7 |
| 1951 | 49 | 1,314,944 | 444,935,001 | 23.82 | 126 | 4,070,478 | 401,452,796 | 88.8 |

[a] Percentage calculated from grain used, based on an assumed average yield of 4.9 proof gal. per 56 lb. bushel of grain.

Source: U. S. Treasury Dept. Annual Report of the Commission of Internal Revenue.

TABLE 2. KIND AND QUANTITY OF RAW MATERIALS USED FOR THE PRODUCTION OF ETHYL ALCOHOL IN THE UNITED STATES

| Year ended June 30 | Molasses gal × $10^3$ | Grain (bu × $10^3$) 1939-1941, Grain (lb × $10^3$) 1942-1950[a]: Corn | Malt | Rye | Other[b] | Ethyl sulfate gal × $10^3$ | Pineapple juice gal × $10^3$ | Other materials[c] gal × $10^3$ | Other materials[c] lb × $10^3$ |
|---|---|---|---|---|---|---|---|---|---|
| 1939 | 163,223 | 18,087 | 3,275 | 5,495 | 61 | 35,389 | 1,594 | 26,177 | 1,254 |
| 1940 | 198,929 | 18,911 | 3,330 | 5,614 | 42 | 45,786 | 3,256 | 13,801 | 12,644 |
| 1941 | 225,349 | 23,347 | 4,126 | 6,679 | 45 | 53,400 | 1,831 | 2,365 | 18,563 |
| 1942 | 286,174 | 2,236,469 | 333,155 | 384,190 | 114,806 | 71,259 | 1,988 | 2,859 | 25,907 |
| 1943 | 183,039 | 2,797,530 | 558,854 | 117,008 | 2,370,391 | 78,299 | 3,237 | 3,574 | 37,136 |
| 1944 | 263,889 | 320,126 | 727,200 | 217,398 | 6,393,230 | 94,467 | 4,918 | 8,680 | 70,030 |
| 1945 | 242,786 | 1,600,028 | 962,002 | 651,649 | 6,464,941 | 91,621 | 2,766 | 57,949 | 97,106 |
| 1946 | 117,520 | 1,370,401 | 537,373 | 463,822 | 1,968,362 | 103,306 | 2,374 | 152,899 | 648,435 |
| 1947 | 73,382 | 2,651,731 | 455,336 | 237,166 | 148,398 | 106,457 | 2,880 | 222,581 | 983,782 |
| 1948 | 178,502 | 1,583,653 | 344,099 | 368,989 | 617,599 | 109,245 | 4,909 | 249,189 | 274,026 |
| 1949 | 161,790 | 1,716,278 | 382,247 | 375,936 | 233,767 | 131,819 | 2,566 | 249,208 | 1,325,285 |
| 1950 | 131,327 | 1,523,057 | 240,216 | 272,288 | 53,902 | 132,237 | 3,331 | 208,850 | 72,816 |

[a] Conversion factor used, 56 lb per bu, when reported in bushels.

[b] Wheat, barley, rice, milo, etc.; also mixtures of corn, malt, and rye.

[c] Diamalt, sulfite liquor, manioc meal, maguey juice, maple sirup, cider, corn sirup, potatoes, crude-alcohols mixture, cellulose pulp and chemical mixtures, etc.

Source: Treasury Dept., Bureau of Internal Revenue, Annual and Special Reports of Commissioner.

alcoholic fermentation of molasses, of sulfite waste liquor, and of wood waste are discussed in Chapters 3, 4 and 5 of this book.

The fermentation of starchy materials, such as grains, is somewhat more involved than that of saccharine materials. This is due to the nature of the raw material and the necessity of saccharification of the starch to fermentable sugars. However, the principles of the fermentation proper and of the fundamental plant operations are similar for all alcoholic fermentations regardless of substrate. These principles are, therefore, discussed in considerable detail in this chapter, and in the subsequent chapters on the alcoholic fermentation of other substrates, they will not be considered in such detail. However, modifications and innovations required by the specific substrates will be emphasized in the latter discussions.

## RAW MATERIALS

It will be seen by reference to Table 2 that the grains most commonly employed are corn, malt (primarily barley malt), and rye. During the latter part of World War II and the early postwar period, large quantities of wheat were used and sorghum grains (milo and kafir) to a lesser extent. Potatoes were also used in sizable quantities and these are included under "other" materials in Table 2.

Table 3 contains data on representative yields of alcohol from different types of grains. These data are from several sources and where possible, the starch content and fermentation efficiency (plant basis) are shown.

Alcohol yield and fermentation efficiency are expressed in the industry in various ways. Since the terms "yield" and "efficiency" will be used frequently, their explanation may be helpful. Efficiency and alcohol yield are usually defined as follows:

$$\%\ \textit{fermentation efficiency} = \frac{\text{actual alcohol produced}}{\text{theoretical alcohol from sugar fermented}} \times 100$$

$$\%\ \textit{plant efficiency or fermentation efficiency (plant basis)} = \frac{\text{actual alcohol produced}}{\text{theoretical alcohol from total carbohydrate used}} \times 100$$

$$\textit{alcohol yield} = \frac{\text{gallons of alcohol of given concentration}}{\text{per standard unit of raw material to process}}$$

TABLE 3. ALCOHOL YIELDS FROM SEVERAL GRAINS

| Kind | Type | Starch content %, dry basis | Alcohol yield PG per bu dry basis[a] | Fermentation efficiency plant basis[b] % |
|---|---|---|---|---|
| Corn[c] | Yellow dent hybrid | —— | 6.00 | 95.5 |
| Corn[c] | Yellow dent hybrid | —— | 5.93 | 94.3 |
| Corn[c] | Yellow dent hybrid | —— | 5.87 | 93.2 |
| Corn[d] | Yellow dent hybrid | 67.4 | 6.00 | 91.9 |
| Wheat[d] | Winter, soft white | 72.5 | 5.78 | 82.5 |
| Wheat[d] | Winter, white club | 72.3 | 5.75 | 82.4 |
| Wheat[d] | Hard red winter | 69.1 | 5.69 | 85.2 |
| Wheat[d] | Soft red winter | 68.4 | 5.60 | 84.6 |
| Wheat[d] | Hard red winter | 62.3 | 5.46 | 90.5 |
| Wheat[d] | Red durum | 62.5 | 5.37 | 89.0 |
| Wheat[d] | Hard red spring (Dark northern spring) | 63.4 | 5.22 | 85.0 |
| Rye[e] | | —— | 4.68[f] | 91.5 |
| Granular wheat flour[g] | | —— | 6.16 | 84.0 |
| Sorghum grain[h] | Milo-Bonita | 68.8 | 6.04 | 93.4 |

[a] PG per bu = gallons of 100° proof alcohol per 56 lb distillery bushel.
[b] Based on total starch charged to process.
[c] Kolachov.[28]
[d] Stark, Kolachov, and Willkie.[40]
[e] Willkie, Kolachov, and Stark.[52]
[f] As received basis.
[g] Erb and Hildebrandt.[19]
[h] Packowski, Adams, and Stark.[31]

The amounts of alcohol produced can be determined by analyses or from inventory records after distillation.

Each of these methods of analyzing a single fermentation or group of fermentations has its own particular value in the industry. Whereas the accountants and management are primarily concerned with alcohol yield, since it reflects the return of salable product per unit of raw material, the technical staff must not overlook efficiency. Fermentation efficiency is an index of the physiological condition of the yeast. Plant efficiency is a standard for the evaluation of all process operations from the raw material through fermentation, or through distillation if based on the alcohol in storage tanks.

Yeast fermentations of grain mashes ordinarily result in fermentation efficiencies of 98% plus or minus 2% (analytical error) on the sugar fermented and the plant efficiency (starch charged to process) will vary with the grain fermented and the method of starch analysis (see Table 3). Alcohol yields have generally been reported

as proof gallons per bushel of grain (as received basis) in commercial practice. The standard distillery bushel is 56 lb. and a proof gallon is one U. S. wine gallon of ethyl alcohol at 100° U. S. proof, or 50% by volume. The trend in the recent literature is to report alcohol yields as proof gallons per bushel of grain (dry basis), or as proof gallons per 100 lb of dry grain and with the starch analysis of the grains which permits a calculation of efficiency.

## CULTURES AND THEIR MAINTENANCE

The alcoholic fermentation of grain mashes is accomplished by means of true yeasts. Those most commonly used are strains of *Saccharomyces cerevisiae;* strains of *Saccharomyces ellipsoideus* or "wine yeasts" may also be used. The criteria of good distillery yeasts are rapid growth, high alcohol and sugar tolerance, efficiency in the conversion of the carbohydrates of grain mashes to alcohol, a maximum growth temperature of at least 90°F, and general hardiness to fairly extreme changes in environmental conditions, such as pH, temperature, and osmotic pressure. Fortunately, a relatively high percentage of yeast strains meets these criteria. Satisfactory fermentation will be obtained with yeast isolated from any commercial bakers' yeast; in fact, some grain-alcohol plants have been operated with bakers' yeast charged directly to the fermentors, although this is not a recommended practice.

There are definite and measurable variations between yeast strains in alcohol yield, effect on the flavor of distillates, alcohol tolerance, and other essential characteristics. For these reasons, it is good practice to select yeast strains in a scientific manner.

The selection of suitable strains may be accomplished by means of indirect physiological studies of alcohol tolerance,[24,25] sugar tolerance, and growth and fermentation rates. A more direct and time-saving practice is to conduct laboratory test fermentations on grain mashes.[39] This results in an accurate evaluation of the yeast strain under simulated plant conditions.

The alcohol tolerance of the yeast is one of its most important characteristics. The maximum alcohol concentration at which growth will occur is of less importance than the effect of lower alcohol concentrations on the fermentation rate. It is impractical to ferment at carbohydrate concentrations equivalent to alcohol at the maximum tolerance of the yeast strain, since the growth and fermentation rate is negligible at that point. Therefore, it is

desirable to determine the fermentation rate of yeast strains at various alcohol concentrations, using a method similar to that of Gray.[25] Table 4 presents data from his work and also lists a few strains of distillery yeasts.

TABLE 4. ALCOHOL TOLERANCE OF CERTAIN DISTILLERS' YEASTS

| *Yeast Culture* No.[a] | Name | Alcohol tolerance[b] % by weight alcohol |
|---|---|---|
| 2 | *Saccharomyces cerevisiae* Hansen | 5.79 |
| 22 | Fission type, unclassified | 5.79 |
| 19 | *Zygosaccharomyces soja* B | 4.82 |
| 1[c] | *Saccharomyces cerevisiae* Hansen | 7.72 |
| 26 | *Schizosaccharomyces mellacei* Jorgenson | 7.72 |
| 24 | *Saccharomyces ellipsoideus* Hansen | 9.65 |
| 25 | *Schizosaccharomyces pombe* | 8.68 |
| 29[c] | *Saccharomyces cerevisiae* Hansen—Rasse XII | 8.68 |
| 4[c] | *Saccharomyces cerevisiae* Hansen | 8.68 |
| 3[c] | *Saccharomyces cerevisiae* Hansen | 10.61 |
| 28[c] | *Saccharomyces cerevisiae* Hansen—Rasse M | 10.61 |
| 31[c] | *Saccharomyces cerevisiae* Hansen | 11.58 |

[a] Seagram stock culture collection number.
[b] Defined by Gray as "the maximum percentage of alcohol (by weight) at which percentage of glucose utilization is no more than 1% below the percentage of glucose utilization in the control flask of the same series" (under the conditions of his test).
[c] Commercially used strains.
Source: Gray.[25]

Cultures of yeast for plant use are usually maintained in the laboratory on malt extract-agar slants. Stock cultures are commonly transferred at monthly intervals and, after incubation to secure good growth, are stored in a refrigerator.

## FERMENTATION MECHANISMS

The yeasts are not able to ferment starch directly, therefore, an essential step in the production of alcohol from grain is the saccharification of the starch to maltose or glucose by means of enzymes or acids. Almost all distilleries now use malted barley (distillers' barley malt) as a source of amylases for this step.

The physical, chemical, and biological transformations that are involved in the conversion of grain to ethyl alcohol may be summarized in four separate stages: (1) hydration of starch, (2) gelatinization of starch, (3) enzymic (or acid) hydrolysis of starch

to fermentable carbohydrates, (4) conversion of sugars to ethyl alcohol by yeast fermentation.

The hydration of starch is facilitated by milling the grain. The finely divided starch becomes sufficiently hydrated on dispersion in water.

The gelatinization of starch is governed by the type of the starch, time-temperature relations, particle size, and mash concentration. The optimal relationships will vary with each type of starch. This step is an essential precursor to the enzymic hydrolysis of starch.

The enzymic hydrolysis or conversion of starch produces a mixture of maltose and unfermentable dextrins. The reaction will reach equilibrium with the maltose-dextrin ratio controlled by the chemical composition of the starch. Starch is composed of amylose and amylopectin. The amylose, or straight-chain starch fraction, is almost quantitatively hydrolyzed to maltose, whereas the amylopectin, or branched-chain fraction, is only partially hydrolyzed.[7] In the case of corn starches, the reaction stops with approximately 80% of the total starch converted to maltose and the remainder to branched-chain fragments referred to as residual or limit dextrins. The dextrins are subsequently slowly hydrolyzed to maltose during the fermentation step. This will be discussed later in more detail.

Sugars can be utilized as a source of energy by almost all animal and plant organisms. With many microorganisms, sugars are a preferred source of energy, but there is a vast difference among various microorganisms as to the type of sugars that can be utilized. Yeasts ferment the D- or naturally occurring forms of glucose, mannose, fructose, and galactose. None of the ordinary yeasts ferments pentoses. The disaccharides sucrose and maltose are also readily fermented by yeasts since they produce the enzymes sucrase (invertase) and maltase which convert these sugars to the fermentable hexoses. There is experimental evidence that maltose may also undergo direct fermentation. Whether this is generally true or maltose is usually first hydrolyzed to glucose is of theoretical interest only.

The over-all reaction of the fermentation of hexose sugar by yeast is expressed by the Gay-Lussac equation:

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2$$

This equation is the basis for all efficiency calculations. However, it has been recognized since the time of Pasteur that minor products

are also regularly produced from sugars by yeasts, including glycerol, lactic acid, and succinic acid.

A great deal of research in many laboratories has been devoted to investigating the metabolism of sugar by yeast and, as a result, the mechanism of the complex transformations which take place and the enzymes responsible for these changes are now better understood than for any other organism. The reactions of yeast fermentation and their interrelationships, usually designated the Embden-Meyerhof-Parnas scheme, are shown in Figure 1.

It is beyond the scope of this book to discuss the details of these reactions or the mass of experimental evidence which has led to the elucidation of this mechanism of yeast fermentation. Excellent treatises, such as those of Baldwin,[9] Porter,[33] Stephenson,[42] and Werkman and Wilson[50a] discuss the mechanism of this and other fermentations in detail and should be consulted by the interested reader.

## ALCOHOLIC GRAIN FERMENTATION PROCESS

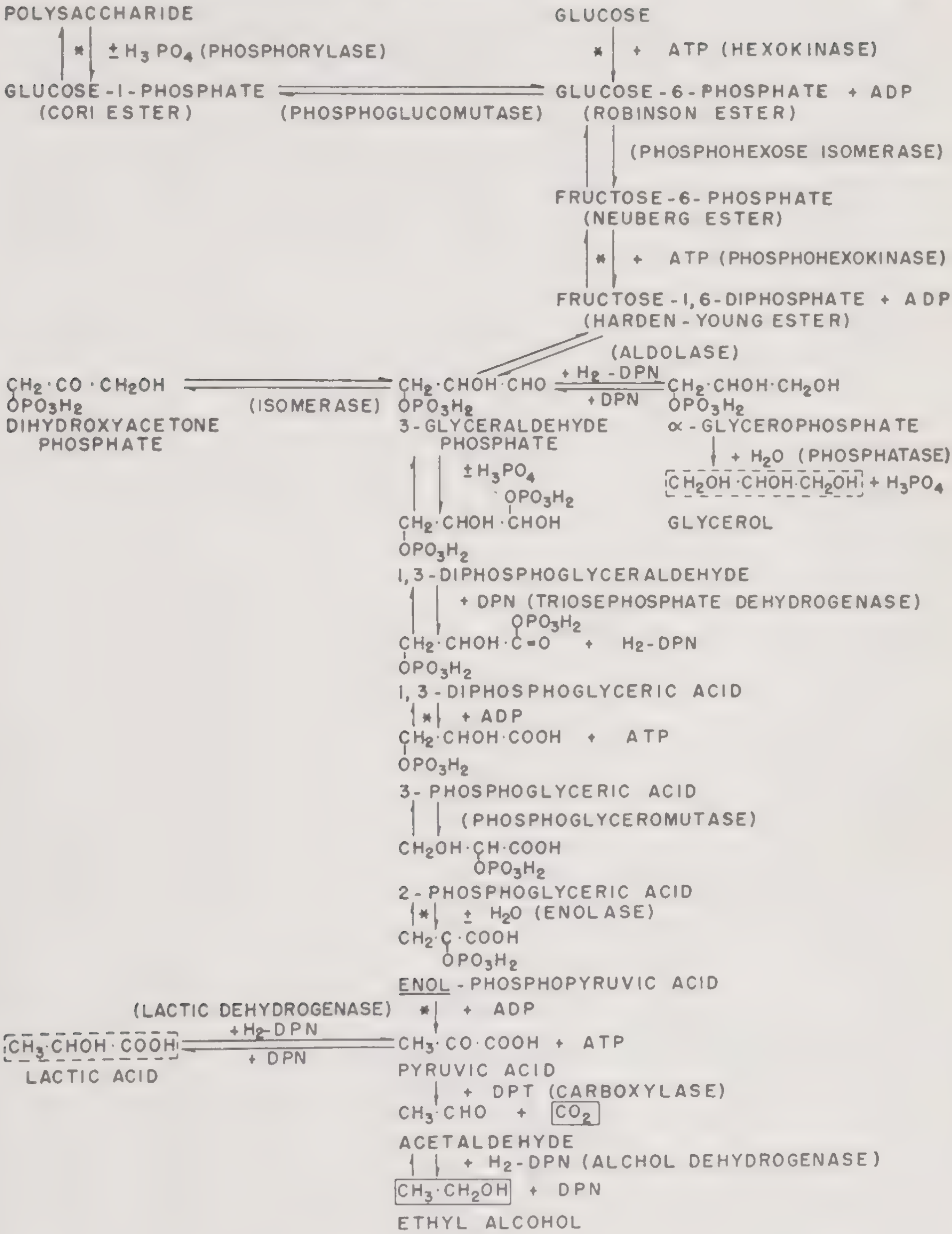
The commercial process of alcoholic grain fermentation has been developed, as is true for all commercial processes, to provide proper conditions for the conduct of the essential reactions on an economic scale and in an economic manner. The essential reactions have been listed before. These are accomplished in commercial practice in the clearly defined and separate, yet integrated, process steps as follow:

(1) Milling—reduction of particle size prior to hydration
(2) Cooking—hydration and gelatinization of starch
(3) Conversion—enzymic hydrolysis of starch
(4) Fermentation—production of ethyl alcohol from fermentable sugars (includes limit dextrin conversion)
(5) Distillation—product recovery
(6) Recovery of grain residues—by-product operation

The details of these operations are discussed in this section.

### Composition of Grain Mashes; Grain Bills

A grain mash consists of two or more kinds of grains mixed with water. The only exceptions to this are the rarely used 100% malt (rye, barley, or wheat) mashes. The relatively high cost of malted grains and their low starch content restrict their use to




* $MG^{++}$ OR $MN^{++}$ IONS REQUIRED.

ATP = ADENOSINE TRIPHOSPHATE

ADP = ADENOSINE DIPHOSPHATE.

$H_3PO_4$ = INORGANIC PHOSPHATE.

DPN = DIPHOSPHOPYRIDINE NUCLEOTIDE (COZYMASE)

$H_2$-DPN = REDUCED DIPHOSPHOPYRIDINE NUCLEOTIDE.

DPT = DIPHOSPHOTHIAMINE (COCARBOXYLASE).

FIGURE 1. *Embden-Meyerhof-Parnas Scheme for Yeast Dissimilation*

the amount that is required as an adequate source of amylases for starch conversion. The other grains supply starch. Industrial-alcohol producers will be guided solely by unit cost, whereas the beverage distillers vary the composition to produce distillates of varying flavors under the existing government regulations. The composition of the mash is known as the "grain bill." Typical grain bills appear in Table 5. The grain bill for any single fermentor contains all grain that is charged to the fermentor, including the grain in the yeast mash.

TABLE 5. TYPICAL GRAIN BILLS; THE COMPOSITION OF THE GRAIN PORTION OF MASHES BY WEIGHT PERCENTAGE

| Type of mash | *Percentage of Total Grain by Weight (as Received)* Corn | Wheat | Milo | Rye | Barley malt | Rye malt |
|---|---|---|---|---|---|---|
| *Spirits*[a] | | | | | | |
| Corn | 90–92 | – | – | – | 8–10 | – |
| Wheat | – | 90–92 | – | – | 8–10 | – |
| Milo | – | – | 90–92 | – | 8–10 | – |
| *Whiskey* | | | | | | |
| Rye | – | – | – | 88–92 | 8–12 | – |
| Rye | – | – | – | 88–92 | – | 8–12 |
| Bourbon | 60–75 | – | – | 13–30 | 10–12 | – |
| Rye excess | 39 | – | – | 51 | 12 | – |
| Corn | 81–90 | – | – | 0–9 | 10–12 | – |

[a] Industrial alcohol or beverage spirits.

## Grain Handling and Milling

Whole grain is received at the distillery by rail or truck. It is weighed, unloaded, cleaned, and conveyed to whole-grain storage bins. The cleaning operation removes dirt, dust, foreign matter, and most of the cracked grain.

The whole grain is ground in mills to reduce it to the proper particle size. Three types of mills are in common use: roller, hammer, and attrition mills. Each type has certain advantages and certain disadvantages. The mills are adjusted to provide the desired particle size for the kind of grain and type of process. Screen analyses of the ground grain are made with United States standard screens. Typical sieve analyses are presented in Table 6 for corn, milo, rye, wheat, granular wheat flour or Alcomeal (a

TABLE 6. PREFERRED SCREEN ANALYSES FOR GRAIN GROUND FOR DISTILLERY PROCESSING[a]

*CORN*

| Cooking process | Continuous | Pressure-batch | Atmospheric-batch |
|---|---|---|---|
| Cooking temperature | 360°F-90 sec | 308°F | 212°F |
| Screen analysis | % | % | % |
| +12 | 24 | 24 | 4 |
| +20 | 52 | 52 | 36 |
| +60 | 20 | 20 | 52 |
| −60 | 4 | 4 | 8 |

*RYE*

| Cooking process | Continuous | Continuous | Pressure-batch | Infusion-batch |
|---|---|---|---|---|
| Cooking temperature | 280°F-3 min | 145°F-20 min | 280°F | 145°F |
| Screen analysis | % | % | % | % |
| +12 | 4 | 4 | 4 | 4 |
| +20 | 36 | 36 | 36 | 36 |
| +60 | 50 | 50 | 50 | 50 |
| −60 | 10 | 10 | 10 | 10 |

*MILO*

| Cooking process | Continuous | Pressure-batch | Atmospheric-batch |
|---|---|---|---|
| Cooking temperature | 350°F-90 sec | 280-308°F | 212°F |
| Screen analysis | % | % | % |
| +12 | 15 | 15 | 4 |
| +20 | 41 | 41 | 52 |
| +60 | 38 | 38 | 38 |
| −60 | 6 | 6 | 6 |

*WHEAT*

| Cooking process | Continuous | Pressure-batch | Infusion-batch | |
|---|---|---|---|---|
| Cooking temperature | 350°-90 sec | 280-308°F | 155°F | |
| Mill | | | Attrition | Roller |
| Screen analysis | % | % | % | % |
| +12 | 4 | 4 | 0 | 4 |
| +20 | 52 | 52 | 29 | 36 |
| +60 | 36 | 36 | 52 | 50 |
| −60 | 8 | 8 | 19 | 10 |

*GRANULAR WHEAT FLOUR OR ALCOMEAL*

| Cooking process | Continuous | Infusion-batch |
|---|---|---|
| Cooking temperature | 285°F-90 sec | 145°F |
| Screen analysis | % | % |
| +12 | 1 | 1 |
| +20 | 26 | 26 |
| +60 | 49 | 49 |
| −60 | 24 | 24 |

*BARLEY MALT OR RYE MALT FOR CONVERSION*

| Screen analysis | % |
|---|---|
| +12 | 4 |
| +20 | 36 |
| +60 | 50 |
| −60 | 10 |

[a] All grain ground with roller mills except where noted. Data, courtesy of Joseph E. Seagram & Sons, Inc., Louisville, Ky.

commercial name applied to granular wheat flour after its introduction during World War II), barley malt, and rye malt. It will be observed that the grind may vary with the cooking procedure. The use of higher temperature for cooking for any given grain permits the use of a coarser grind, as will be seen from the data on corn, milo, and wheat. Malt is never subjected to temperatures higher than 145°F in order to minimize amylase destruction and thus a relatively fine grind is employed to facilitate the extraction of amylases. Similarly, small grains mashed by the infusion process are usually ground more finely than when the same grains are pressure cooked; this is the case with wheat, but not with rye. Every effort is made to avoid a high percentage of flour in the grind, since the fine particles may burn before the larger particles are thoroughly gelatinized, thus reducing alcohol yield. Variations in milling practices will be found dependent on the type of mill, problems of recovery of grain residues, and many other factors.

The ground grain is conveyed to bins where it is held until charged to process through scale hoppers. Ground-grain storage has been eliminated in some plants employing continuous cooking systems which permit a continuous flow from whole-grain storage to the fermentors.

A detailed discussion of the problems and methods of grain handling and milling for distillery use is given by Willkie and Prochaska.[53]

## Cooking of Grain Mashes

It is essential that the starch in the ground grain is thoroughly gelatinized to permit enzymic hydrolysis with the barley-malt amylases prior to the yeast fermentation. The extent of gelatinization is governed by the time and temperature relationship and particle size, and uniform distribution of heat is essential. The control of pH is of minor importance and while there are some indications that a pH of 5.4 to 5.6 is preferable, the natural pH values of grain mashes (5.5 to 6.0) are satisfactory. As in most industrial processing, the final process represents an economic compromise with as close an approach to the ideal conditions as may be achieved.

There are four basic cooking processes in commercial use:

(1) the batch-infusion process; (2) the batch-atmospheric process at 212°F; (3) the batch-pressure process, using temperatures up to 308°F; and (4) continuous-pressure cooking. Continuous-infusion processes for small grains are in development.

The starches of small grains are susceptible to gelatinization at low temperatures; for instance, wheat and granular wheat flour may be gelatinized sufficiently at 155°F in 30 minutes and rye at 145°F in 30 minutes or less. Corn starch is evidently gelatinized in two steps, approximately 80 to 85% at 160° to 165°F and the remainder only as the temperature is increased, with the final 2 to 5% requiring treatment at temperatures above 212°F and up to 350°F, depending on the reaction time. Since corn is the most widely used raw material, almost all distillers employ either batch-pressure or continuous-pressure cooking systems to attain the maximum yield of alcohol.

### Infusion Process

This is probably the oldest method still in use and is only suitable for small grains. The temperature range permits cooking and conversion to occur simultaneously.

The equipment consists of a horizontal or vertical mash tub equipped with a mechanical agitator and a steam sparger. The capacity may be as much as 10,000 gal. At a temperature of 100° to 110°F, 22 to 28 gal of water per bushel of grain is drawn. Ground grain weighed by means of a scale hopper is added with agitation and at a rate that will minimize the formation of "dough-balls." The temperature is raised at the rate of 1°F per minute to 155°F for wheat mash or granular wheat-flour mash, held 45 minutes, cooled to 145°F, malt added, held 5 to 30 minutes, and the mash is then cooled. When rye is mashed, the malt is added first and the maximum temperature is 145°F. A usual variation when mashing rye is to hold for 15 minutes at 130°F to facilitate proteolytic action and then raise the temperature to 145°F. This tends to alleviate foaming, a serious problem in the fermentation of rye mashes. There are minor variations of these conditions in different plants. Another operating procedure that is in commercial use for the batch-infusion mashing of rye grain is illustrated in Figure 2. It will be noted that the conditions are similar to those described here. The total processing time for each batch varies from 115 to 135 minutes.

The principal disadvantages of the infusion method are ex-

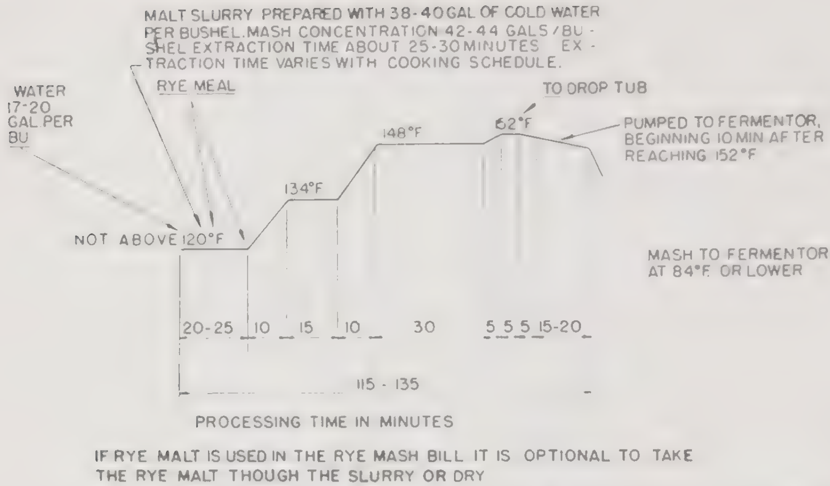



FIGURE 2. *Batch-Infusion Mashing of Rye* (Courtesy—Hiram Walker & Sons, Inc., Peoria, Ill.)

tensive equipment requirements for a given plant capacity and a barely adequate reduction in the total number of bacterial contaminants in the mash. Although good alcohol yields are obtained from rye, malt, and wheat mashes prepared by this process, the frequency of low yields caused by bacterial contamination is much higher than is experienced with mashes cooked under pressure.

### Batch-Atmospheric Process

This process is used almost exclusively for corn or milo. Rye and wheat mashes, cooked at atmospheric pressure at 212°F, foam badly and the alcohol yields are much lower than those obtained with either the infusion or pressure-cooking processes. The equipment is essentially the same as is used for the infusion method, in fact, they are used interchangeably in many plants. The cooking conditions are similar to those shown in Table 7 for batch pressure cooking except that the maximum temperature range is 208° to 212°F and the time is 60 minutes at these temperatures. The cooking or gelatinization is incomplete as evidenced by alcohol yields 4 to 5% lower than those obtained from the same grains when pressure cooked. The process is obsolete although still in use in some of the smaller plants.

### Batch Pressure Process

The conventional batch pressure-cooking system, still widely used in the distilling industry, consists of a number of horizontal

TABLE 7. CONDITIONS OF BATCH PRESSURE COOKING AND CONVERSION

| | | | |
|---|---|---|---|
| | *COOKING* | | |
| *Type of grain* | *Corn* | *Rye* | *Wheat* |
| Temperatures, °F | | | |
| Initial | 125 | 100 | 122 |
| Maximum | 305 | 295 | 305 |
| Final | 152 | 152 | 152 |
| Time, minutes | | | |
| At maximum temperature | 0–10 | 5–15 | 10–15 |
| Total cycle, hours | 3 | 4 | 3 |
| Mash concentration, gallons of water per bushel | 22.0 | 28.0 | 21.0 |
| pH | 5.6 | 5.6 | |
| | *MALT-SLURRY PREPARATION* | | |
| Temperature, °F | 90–100 | 90–100 | 90–100 |
| Mash concentration, gallons of water per bushel | 35–40 | 35–40 | 35–40 |
| Time, minutes | 5 | 5 | 5 |
| Quantity of malt, % | 8–10 | 10–12 | 8–10 |
| | *CONVERSION* | | |
| Temperature, °F | 145 | 145 | 145 |
| Time, minutes | 30 | 30–60 | 5 |
| Mash concentration, gallons of water per bushel | 30–32 | 36–38 | 30 |

NOTE: No information is available on batch pressure cooking of milo, but conditions for wheat should apply to milo.

Data, courtesy of Joseph E. Seagram & Sons, Inc., Louisville, Ky.

pressure vessels up to 10,000 gal in capacity, or larger, designed to operate at 100 psi gage pressure. These cookers have a horizontal agitator of the rake type and steam jets along the bottom. The number and size of the cookers vary with the plant capacity. Figure 4 illustrates grain handling, batch pressure cooking and batch conversion. The cooking cycle and conditions for corn, rye, and wheat are shown in Table 7. The necessary amount of water is added in the cooker, grain charged, the temperature raised to the maximum and held for the required time. The pressure is then blown down to atmospheric and vacuum is applied to cool the mash to 150°F by evaporation. The addition of the malt slurry at 100°F causes the temperature of the mixture to drop to 145°F. This is then pumped to the converter for batch conversion.

Figures 5 and 6 illustrate the batch pressure cooking of corn and milo mashes and of wheat mash respectively under somewhat


FIGURE 3. *Horizontal Batch Pressure Cookers* (Courtesy—Hiram Walker & Sons, Inc., Peoria, Ill.)

different conditions than those given in Table 7. The cooking cycle is shown graphically and the conditions are given in detail. The information of Table 7 and Figures 4 and 5 has been provided by two different members of the industry and is illustrative of the variations in operating procedures that are found in the industry. These variations are a reflection of equipment differences and local plant conditions, which make it desirable for each plant to develop optimal conditions that will be compatible with their own equipment and over-all operation. Each of the previously described procedures for each kind of grain will result in comparable alcohol yields in the plant for which the procedure has been developed.

The principal advantage of this pressure-cooking system over those described previously is a 4 to 5% higher alcohol yield from corn and milo and a higher average alcohol yield for wheat and rye, due to the elimination of bacterial contamination in the cooked mash. The average yield increase with wheat and rye may be as

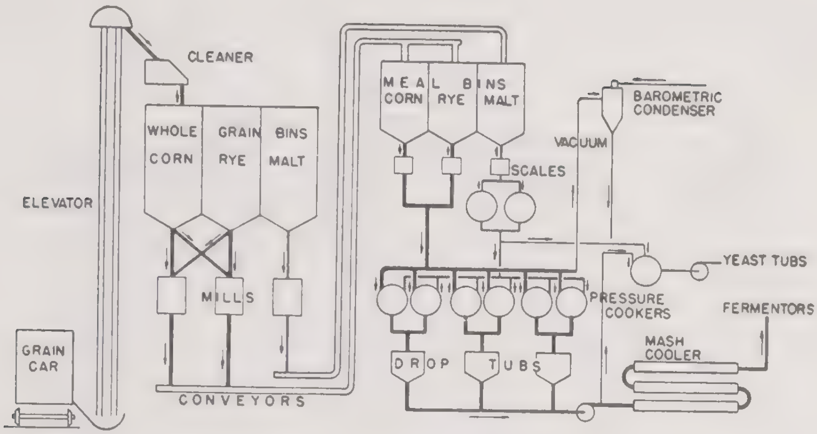



FIGURE 4. *Distillery Batch-Cooking System* (Courtesy—Joseph E. Seagram & Sons, Inc., Louisville, Ky.)

great as 0.4 proof gallons per bushel, or approximately 8 to 9%. The disadvantages are high over-all steam consumption, uneven and heavy peak loads on the power house, manual control and the attendant added labor cost, and a relatively high capital investment in equipment and building space. Many of these disadvantages also apply to the batch atmospheric and infusion processes.

**Henze Cooker.** The Henze cooker is a modified batch pressure cooker designed to cook potatoes. It has been described by Foth.[21] The cooker is vertical with a steep conical bottom and large bottom outlet valve. The starchy substrate is cooked in the Henze cooker under pressure and, at the end of the cooking cycle, is blown under pressure to a drop tub maintained at atmospheric or reduced pressure. The blow down results in violent boiling and thorough disintegration of the starch granules of the substrate. Some distilleries have used the Henze cooker to cook whole grain, thus eliminating the milling operation, but it has not been widely accepted in distilleries on the North American continent. Recent information indicates that this cooker is not too successful with grain, since its disintegration may become so complete as to interfere with grain by-products recovery. However, at least one plant in the United States regularly cooks whole grain, using conventional horizontal cookers, blowing the cooked grain from the cooker under pressure to a second cooker held under reduced pressure to

secure disruption of the grain kernels, and cooling to conversion temperature.

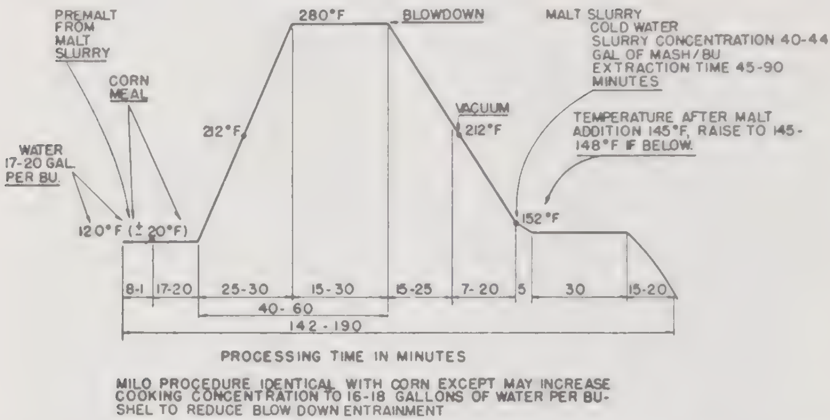



FIGURE 5. *Batch Pressure Cooking of Corn and Milo Mashes* (Courtesy–Hiram Walker & Sons, Inc., Peoria, Ill.)

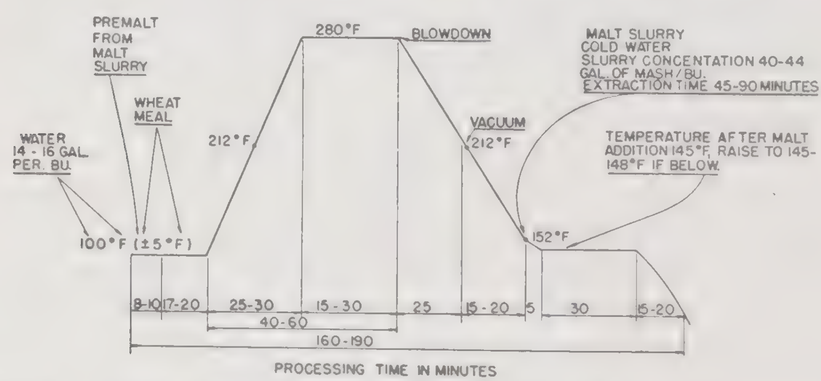



FIGURE 6. *Batch Pressure Cooking of Wheat Mash* (Courtesy–Hiram Walker & Sons, Inc., Peoria, Ill.)

## CONTINUOUS PRESSURE COOKING

The continuous, rapid process for grain cooking is one of the outstanding recent developments in the distilling industry. In fact, this is one of the major engineering developments in the fermentation industry within the last decade. A recent paper by Pfeifer and Vojnovich[31a] describes in some detail the continuous steam sterilization of media for biochemical processes. This is the simplest, most economical, and most efficient method for completely removing contaminating microorganisms from fermentation media.

Pfeifer and Vojnovich describe the pilot-plant equipment used for continuous sterilization at the Northern Regional Research Laboratory and its application in the continuous sterilization of

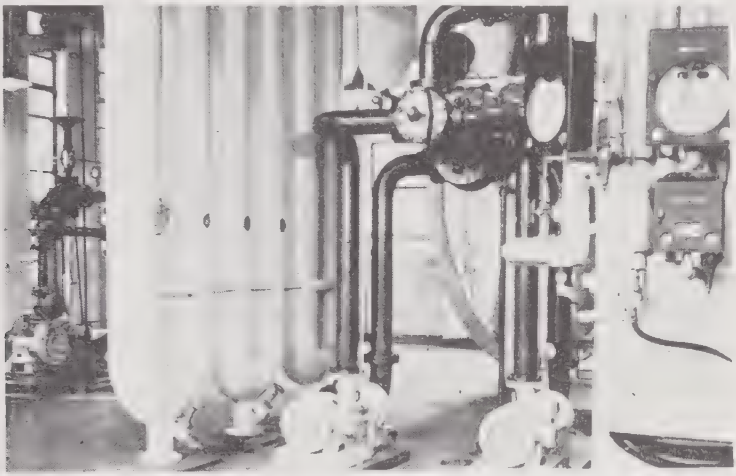


FIGURE 7. *Precooker and U-Tube Portions of the Continuous Cooking System* (Courtesy—Joseph E. Seagram & Sons, Inc., Louisville, Ky.)

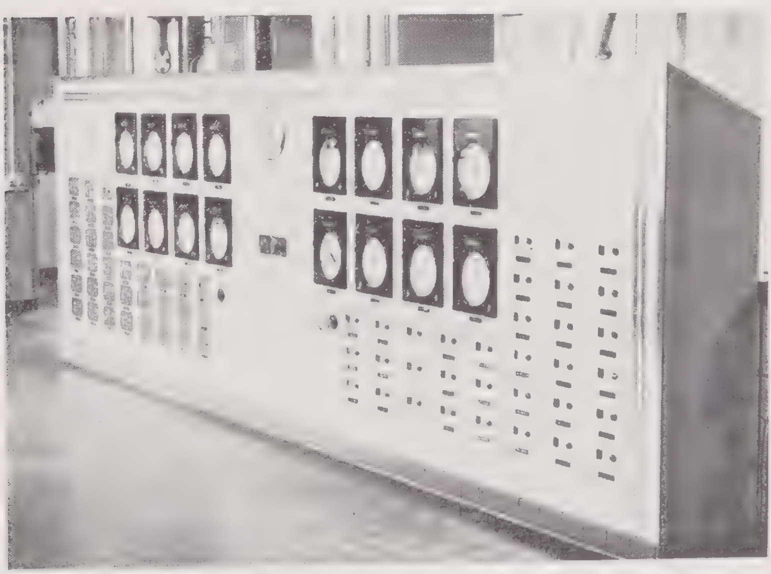


FIGURE 8. *Instrument Control of the Continuous Cooking System is Centered in a Panel Board* (Courtesy—Joseph E. Seagram & Sons, Inc., Louisville, Ky.)

various commercial media for a number of different fermentations. They discuss the design of continuous sterilizers and give some general data obtained from commercial installations, including those for grain mashes. There is no doubt that continuous sterilization equipment will be installed in many new plants for the production of vitamins, antibiotics, enzyme concentrates, and industrial organic chemicals.

In the continuous pressure cooking of grain mashes for the alcoholic fermentation, all milling, cooking, and conversion operations are integrated in the process, as described by Unger, Willkie, and Blankmeyer.[47]

A flow sheet of this system, including the fast conversion process and vacuum mash cooling which are described later, is presented in Figure 9.

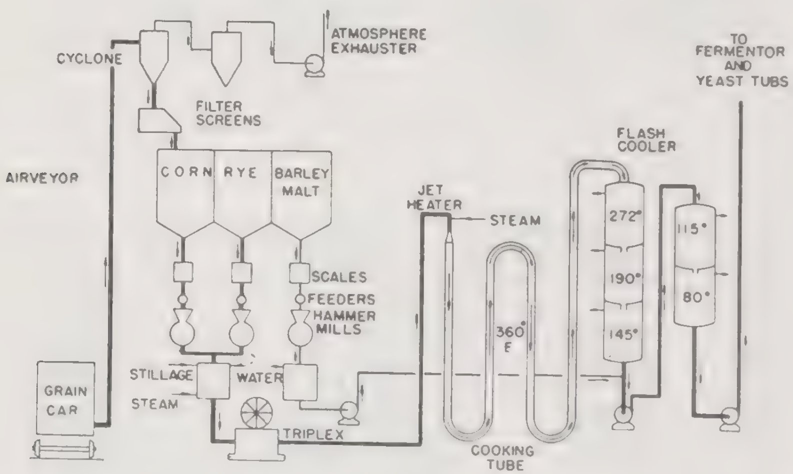



FIGURE 9. *Distillery Continuous Cooking System* (Courtesy—Joseph E. Seagram & Sons, Inc., Louisville, Ky.)

Whole grain is continuously weighed into the mills, discharged, and conveyed to a slurry tank or precooker to which water and stillage, the dealcoholized, fermented beer discharged from the base of the beer still, are continuously metered through a steam-jet heater. The precooker is equipped with an agitator and/or a recirculating pump. An automatic temperature controller maintains the temperature at the desired point by controlling the steam flow to the ejector on the water-stillage line. The retention time in

the precooker is 1 to 5 minutes. From here, the mash is continuously pumped to a steam-jet heater where it is instantaneously heated to the cooking temperature and then passed through a pipe line with several U-bends in series and of sufficient capacity to provide the desired retention time at any given flow rate. The cooked mash is discharged through a relief valve to a flash chamber. The flash steam may be recovered for process use. Mash from this chamber, usually operated at 10 to 15 psi gage pressure, passes to a vacuum flash chamber where it is cooled to a conversion temperature of 145°F.

The continuous pressure-cooking process offers several advantages: uniform cooking, automatic control, uniform load on the power house, lower net steam consumption, and a substantial reduction in capital investment in equipment and buildings. Alcohol yields obtained from mashes cooked by means of this process are equivalent to the best yields from batch pressure cooking.

The operating conditions developed in commercial practice for this process are presented in Table 8 for corn, wheat, rye, and milo. Continuous pressure-cooking systems have been installed in an increasing number of distilleries in the last 10 years.

TABLE 8. CONTINUOUS-COOKING AND FAST-CONVERSION OPERATING CONDITIONS

| | | | | |
|---|---|---|---|---|
| *PRECOOKING* | | | | |
| Temperature | 140–155°F | | | |
| Mash concentration | 20 gal per bu | | | |
| pH (adjusted with stillage) | 5.1–5.4 | | | |
| Time | 1–5 minutes | | | |
| *COOKING* | | | | |
| Type of grain | Corn | Rye | Wheat | Milo |
| Temperature, °F | 360 | 280 | 350 | 350 |
| Time, minutes | 1.5 | 3.0 | 1.5 | 1.5 |
| Mash concentration | Approximately 25 gal water per bu | | | |
| *MALT-SLURRY PREPARATION* | | | | |
| Temperature | 145°F | | | |
| Mash concentration | 40 gal water per bu | | | |
| Time | 1.0 minute | | | |
| Quantity of malt | 8–10% of total grain | | | |
| *CONVERSION* | | | | |
| Temperature | 145°F | | | |
| Time | 2.0 minutes | | | |
| Mash concentration | 25–30 gal per bu | | | |

Data courtesy of Joseph E. Seagram & Sons, Inc., Louisville, Ky.

## Conversion of Grain Starch to Fermentable Carbohydrates

The gelatinized or cooked grain mash is converted to fermentable carbohydrates in all or almost all distilleries on the North American continent by the use of malted grains, most commonly distillers' barley malt, usually specified with a Lintner value of not less than 170° or a Lasche value of less than 7 to 8 minutes.[15] Distillers' malt has a higher diastatic value than brewers' barley malt.

The use of malt with the infusion cooking process has been described. The conversion of atmospheric- or pressure-cooked mashes is accomplished in a separate step by either the batch or the fast continuous conversion process. The process conditions are summarized in Tables 7 and 8, respectively.

### Batch Conversion Process

The batch process (Figure 4) has been used for many years with the batch cooking process. The finely ground barley malt is slurried in water at 90° to 100°F and at a concentration of 35 to 40 gal of water per bu for 5 minutes. It is then added to the cooked mash. The resultant mixture at 145°F is agitated in a mash tank or converter for 30 to 45 minutes and then pumped to the coolers. During this operation, the starch is liquefied rapidly and approximately 75 to 80% is converted to maltose and the remainder to residual dextrins.

### Continuous Fast Conversion Process

A recent improvement in the conversion operation is the fast, continuous process (Figure 6) described by Gallagher, Bilford, Stark, and Kolachov[22] and by Blankmeyer and Stark.[11] Whole malt is continuously weighed into the mills and the ground malt conveyed to a slurry tank. Water, at 145°F, is metered into the slurry tank which provides a retention time of 1 to 5 minutes. The infusion, at a concentration of 40 gal water per bushel, is continuously pumped into the stream of cooked mash at 145°F. The resultant mixture is pumped through a pipe line for 2 minutes at 145°F to provide conversion or with a vacuum mash cooler, the malt slurry is introduced between stages, since sufficient retention time is obtained in the succeeding stages of the cooler. This short time interval has been found adequate to hydrolyze the starch to about 70% maltose and 30% dextrins, with good liquefaction.

The mash then passes to the fermentors through pipe coolers, or is cooled with a vacuum cooler. This conversion process requires much less equipment and building space and, apparently due to the preservation of enzymes for subsequent conversion of residual dextrins to sugar during fermentation, has been shown to result in about 2% increased yields of alcohol as compared with the batch conversion processes.

### Amylo Process

This process should be mentioned since it has been extensively used in European distilleries and is in use in at least one plant in Argentina. The conversion of starch is accomplished by the growth of a mold strain, *Rhizopus delemar*, in the main fermentor mash prior to inoculation with yeast.[20,30] The grain mash is cooked with steam under pressure, some acid being added before cooking to make the mash less viscous. The sterile mash is then cooled to about 40°C (104°F), seeded with a culture of the saccharifying mold, and vigorously aerated for about 24 hours while the mold is developing. The mash is then cooled to 32°C (90°F) and seeded with a suitable yeast culture. High yields of alcohol are obtained with this process. However, equipment requirements and the added process time are factors that have been responsible for nonacceptance in North America.

### Premalting

It is common practice with both the batch atmospheric and the batch pressure-cooking systems to add a small amount of barley malt (10% of the total malt) as "premalt." This is added to the grain-water slurry prior to cooking. The presence of this malt during the initial stages of gelatinization causes sufficient starch liquefaction prior to thermal destruction of the amylases to facilitate handling of the mash during cooking and particularly while cooling to conversion temperature. The use of premalt is not ordinarily practiced with the continuous cooking process.

## Mash Cooling

It is necessary to cool the converted grain mashes from 145°F to the fermenting temperatures of 70° to 85°F. Although this is essentially a physical operation or mechanical step, it is essential and deserves discussion since mash-cooler design has an important and

frequently overlooked bearing on alcohol yield. Improperly designed or operated coolers may become prime sources of bacterial contamination. Water economy and the maximum temperature of cooling water are important engineering factors in the selection of mash coolers for a given plant, but operating sanitation and ease of effective cleaning and sterilization must be given prime consideration.

Surface exchangers of many varieties have been used. These include double-pipe, multitube, spiral, and plate types. The double-pipe exchanger has a distinct biological advantage in that mash flow is in series and positive whereas both the plate and the multitube units employ parallel flow which permits grain solids to lodge and result in pockets conducive to the development of bacterial contaminants. Some lodging has also been encountered with the spiral-type exchanger. Thus, these types require more frequent cleaning and partial disassembly for good results.

Evaporating or vacuum mash cooling has been successfully employed where sufficient low-temperature water is not available.[34] These units operate with a high degree of freedom from bacterial contamination due to serial flow at high velocity, freedom from pockets, and the fact that any mash adhering to the walls of the vessels is almost instantly dehydrated to a high-sugar-content sirup. These units are the latest type of distillery mash cooler introduced in the industry.

## Stillage or Backset Systems

Stillage is added to the cooked and converted grain mash prior to fermentation for several reasons. The stillage lowers the pH to a more favorable value, adds buffering capacity to the mash during fermentation, and furnishes readily available nutrients for the yeast. The amount employed varies from 20 to 25% of the final mash volume. Whole stillage from the base of the beer stills is screened to remove the coarse grain solids and the thin stillage is returned to the cookers or fermentors. If screens are not available, then whole stillage may be used. This practice is known as the use of "backset." Stillage is discharged from the base of the conventional atmospheric beer stills at 220° to 225°F and may be stored hot (over 180°F) until used, although this is not a recommended practice. A few plants use vacuum beer stills which operate

with a base temperature as low as 100°F. These plants either require separate systems for stillage sterilization or may add all of the stillage as part of the water during cooking.

## Fermentation

The fermentation phase of the process includes the preparation of the yeast culture through the laboratory and plant stages, the preparation of the mash for yeast growth, and finally the main fermentation. This part of the plant system is shown in the flow sheet of Figure 10.

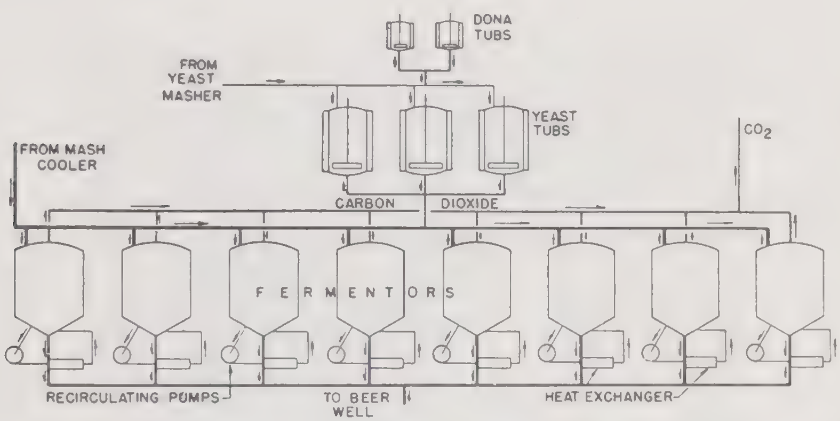



FIGURE 10. *Fermentation and Yeasting Systems* (Courtesy—Joseph E. Seagram & Sons, Inc., Louisville, Ky.)

### YEAST PREPARATION

Preparation of the yeast for inoculation of the fermentors involves both laboratory and plant procedures.

**Laboratory Procedure.** A pure culture of a commercially tested yeast strain is maintained on a suitable medium, usually a malt extract or glucose-yeast extract agar. New stock cultures on agar slants are prepared every 30 to 60 days. An adequate volume of actively growing yeast culture for the first plant stage is prepared by serial transfer in liquid medium, the volume usually necessitating three laboratory transfers. It is customary to use 2 to 5% by volume inoculum and to incubate the first two transfers at 30°C for 24 hours. The final laboratory culture is used after 18 hours of incubation when the culture is at peak activity. It has been found

good practice to maintain daily serial transfers of the culture in liquid medium as a secondary stock from which each culture for plant use is started. Some laboratories prefer to carry the secondary stock through daily transfers for 1 week before use as starter for plant cultures. There is some evidence that the fermenting activity of the yeast culture is enhanced by this means.

The selection of a medium for these laboratory liquid cultures varies from plant to plant. A good medium is 15° Balling malt mash made by the infusion process. The Balling hydrometer scale expresses essentially the percentage of dissolved solids in the mash, 1° Balling representing 1% solids. Commercial malt extract or plant

FIGURE 11. *Propagation of Pure Culture Plant Yeast* (Courtesy—Hiram Walker & Sons, Inc., Peoria, Ill.)

yeast mash that has been strained may be used and the second is generally employed for the final laboratory stage.

**Plant Yeast Procedure.** The number of plant stages required for preparing sufficient yeast to inoculate a plant fermentor depends on the fermentor capacity. Two stages are the most common.

The medium employed is mash prepared at a higher concentration than the fermentor mash. Common practice is to use 16 gal water per bushel of grain and the yeast mash is relatively rich in nutrients as compared with a corn-spirits mash. At one time, either 100% barley malt mashes or 50% rye to 50% barley malt mashes were used. It has been found that a 70% corn to 30% barley malt mash provides sufficient nutrients. Lower levels of malt may be inadvisable depending on other conditions.

The small-grains yeast mash is generally prepared by the infusion process, whereas plants equipped with continuous mashing systems increase the flow rate and concentration of the malt slurry while mash is being pumped to the yeast room. The mash is pumped to the yeast tubs at 130° to 145°F. The pH of the yeast mash is adjusted to 3.6 to 4.0, customarily 3.8, either by the addition of sulfuric acid or by the cultivation of lactic acid bacteria. The first is called "sweet mash" and the second "sour mash." Lactic souring is customary and is accomplished by inoculating the mash with a lactic culture from an actively souring mash or from the laboratory and maintaining the temperature at 128° to 130°F. Mash will sour spontaneously almost as quickly as with inoculum at this temperature, due to lactics of the *Lactobacillus delbruckii* type in barley malt that survive mashing temperatures. However, the use of inoculum does accelerate souring to some extent and reduces the frequency of "gassing sours," probably due to mixed lactics, although frequently assumed to be caused by thermophilic butyrics. The mash is agitated continuously or at frequent intervals to eliminate surface cooling. Acid production is rapid and the initial pH falls from 5.4-5.6 to 3.8 in 6 to 10 hours. The activity of the lactics is stopped by increasing the temperature, otherwise the pH will fall to about 3.5, which is undesirable.

Just prior to adding the yeast culture, the sweet mash or sour mash is pasteurized at 180°F for 30 minutes or it may be held at 212°F or above. It is then cooled to 75° or 80°F and the yeast culture from the laboratory is added. The first plant stage is frequently called the 'dona." The yeast culture develops at a

temperature of 80° to 85°F until the Balling reading has dropped to approximately 50% of the original. The dona is then used to inoculate the main yeast mash and this culture is handled in the same manner. Each of these stages requires 12 to 18 hours, depending on the temperature, agitation, and inoculation ratio. The last is usually 2% by volume. Many plants start a new dona from a laboratory culture only once a week or once a month and rely on "backstocking," inoculation of a new mash with a previously finished yeast mash.

When a fermentor is not ready to receive finished yeast mash, the activity of the yeast may be retarded greatly by cooling at 60° to 65°F. This makes it possible to divide a single finished yeast mash between two or three fermentors, provided the yeast is not more than 24 to 26 hours old and the Balling is 40 to 60% of the original. The ability of a healthy yeast culture to withstand rapid temperature changes and to regain activity quickly on transfer to new mash is another physiological characteristic that is fully exploited in commercial practice.

The equipment used in the yeast departments of distilleries may be open wooden tanks in the older and smaller plants and open steel tanks or closed steel pressure vessels in the more modern plants. The relative freedom from bacterial contaminants in the finished yeast culture in most plants is due less to equipment and aseptic operation of the type practiced in other plants, such as butyl alcohol and antibiotic units, than it is to capitalizing on the physiological differences between yeast and distillery-type contaminants. For example, the low pH increases the effectiveness of pasteurization and inhibits the growth of contaminants, particularly acid formers. The high initial sugar concentration, 22° to 24° Balling (actually about 15% sugar plus dextrins), raises the osmotic pressure to levels inhibitory for most bacteria and when this is reduced by yeast growth, the alcohol content of the finished yeast, approximately 7% by volume, aids in the inhibition of contaminants. Thus, the finished yeast mash is singularly free of bacterial contaminants. This, however, should not be construed to mean that sanitary plant practices are unnecessary.

Recently a new process for the manufacture of pure-culture distillers' yeast[10] has been applied industrially. This process has been described as semiaerobic and one which produces yeast with good storage and fermentation characteristics. The medium for

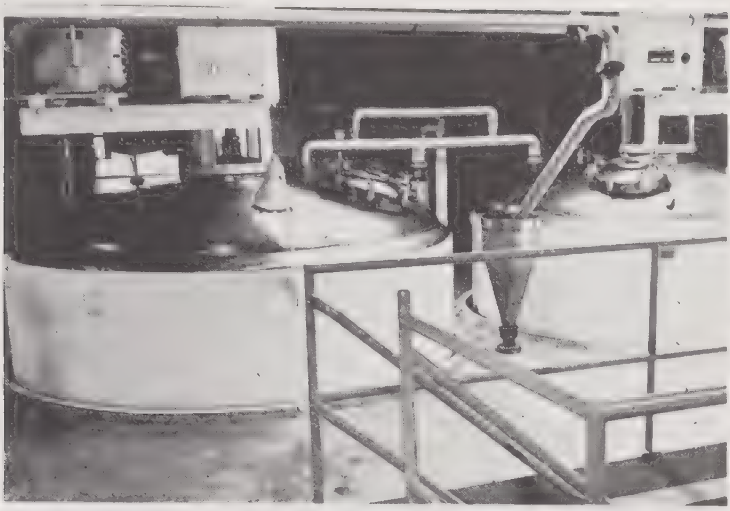


FIGURE 12. *Equipment for Semiaerobic Yeast Production* (Courtesy —Joseph E. Seagram & Sons, Inc., Louisville, Ky.)

yeast propagation consists of a 70% corn—30% barley malt mash, containing 20% stillage by volume. The concentration of the mash is 16° Balling and 0.0053 lb urea per gallon is added as nitrogen supplement. The mash is sterilized under steam pressure, cooled, and inoculated with a pure culture of yeast. Sterile air is introduced at the rate of 1/8 volume air per volume of mash per minute and is dispersed by means of turbine-type agitators. It is reported that a yeast concentration of 400 to 500 million cells per milliliter is obtained after 16 hours of growth at 86°F. Presumably the high concentration of yeast, approximately three to four times the level which is obtained with conventional anaerobic yeast propagation, reduces proportionately the volume of finished yeast which is required for inoculation of the main fermentation.

MAIN FERMENTATION

This represents the culmination of all processing steps up to this point and is relatively simple in itself. As will be discussed later, most of the factors that govern the efficiency of the fermentation and the resultant yield of alcohol occur in processing prior to the final stage. After a fermentor is "set" (filled, inoculated, mash concentration, and pH adjusted), with the exception of temperature control, practically nothing can be done that will alter the final yield of alcohol.

The yeast inoculum is added to the fermentor as soon as 10% or more of the mash volume has been pumped. The final concentration of the mash is adjusted to the desired level, usually 36 gal mash per bushel of grain, equivalent to approximately 13 g total sugar as glucose per 100 ml mash. This will vary for different mash bills and plants and, in the case of rye mashes, the concentration may be as low as 45 gal mash per bushel. This adjustment may be accomplished by dilution with stillage and/or water. In some of the latest continuous mashing and conversion systems, all of the desired stillage and water are added to the precooker and process control is such that the mash enters the fermentor at the desired concentration, thus eliminating final adjustment in the fermentor.

FIGURE 13. *Fermentor Room* (Courtesy—Joseph E. Seagram & Sons, Inc., Louisville, Ky.)

The initial pH of the fermentation is most important, as will be discussed later. This is adjusted to 4.8 to 5.0, with stillage equal to 20 to 25% of the fermentor volume or with sulfuric acid. Mashes with a high buffer capacity may require sulfuric acid in addition to stillage.

The initial or setting temperature depends on whether or not the fermentor is equipped with cooling devices, such as coils, external heat exchanges, or spray rings. In any case, the initial temperature is such that the maximum temperature during fermentation will not exceed 90°F. If no fermentor cooling is available.

the temperature may vary from 66° to 78°F (this is controlled at the mash coolers), is determined by experience, and governed by fermentor volume, mash concentration, and probable atmospheric conditions during the next 48-hour period. Under these circumstances, the distiller pays close attention to weather forecasts. When there are adequate cooling facilities, it is common practice to set at 85°F and control so the temperature does not exceed 90°F.

The fermentation reaches maximum activity between 12 and 30 hours, depending on temperature, agitation, and yeast strain.

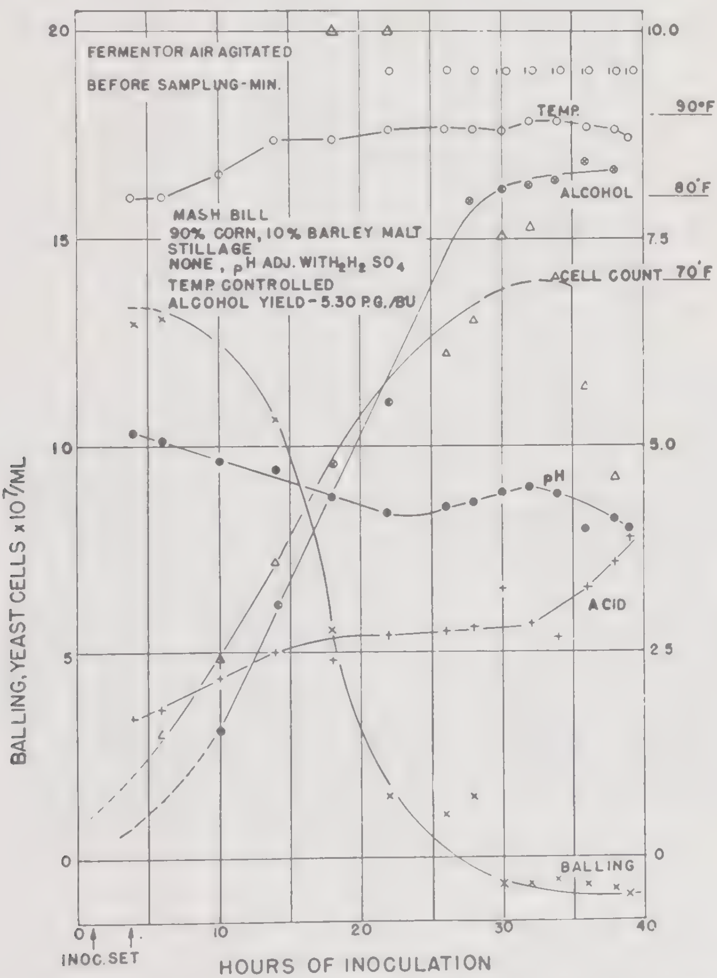



FIGURE 14. *Physical and Chemical Changes during a Typical Alcoholic Fermentation of Grain Mash* (Courtesy—Joseph E. Seagram & Sons, Inc., Louisville, Ky.)

Since the residual dextrins are slowly converted to maltose during fermentation, its time is much more protracted than in most molasses fermentations and it is not complete until 40 to 60 hours after inoculation when temperature is controlled, and 56 to 72 hours without temperature control. After the period of maximum activity, very little reducing sugar is present, since the large, active yeast population metabolizes reducing sugars much faster than the rate of conversion of residual dextrins. The changes that occur during fermentation, such as pH, temperature, reducing sugar, total sugar, and cell count, and alcohol concentration in a typical and normal grain fermentation are illustrated graphically in Figure 14.

### Distillation

The fermented beer, which contains 6.5 to 8.5% alcohol by volume, is pumped from the fermentor to a beer well. The beer well usually has sufficient capacity to hold the contents of approximately two fermentors and is equipped with mechanical agitators to keep the grain solids in suspension. This serves as a feed tank for the distillation system.

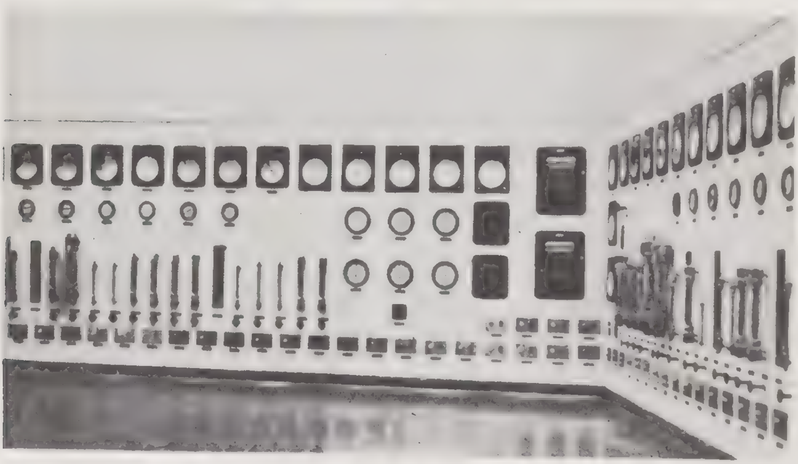


FIGURE. 15. *Control Room for a Distillation System* (Courtesy—Joseph E. Seagram & Sons, Inc., Lawrenceburg, Ind.)

All modern grain-alcohol plants employ continuous stills. The number of columns, complexity of the system, the operating procedures, and the attendant operating efficiencies and costs vary widely with the desired quality of the finished products. Barron[10] describes the theory and practice of alcohol distillation. It should

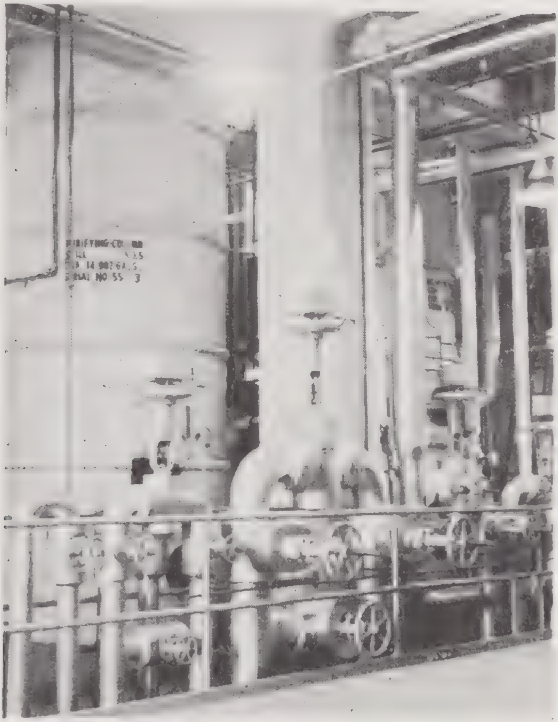


FIGURE 16. *Distillation Columns and Steam-Control Valves of Multi-column Distillation Unit* (Courtesy—Joseph E. Seagram & Sons, Inc., Louisville, Ky.)

be emphasized that improvements in this field have been numerous in recent years.

The fermented mash or beer is pumped into the upper section of the beer still and direct steam enters the bottom. The steam passing up the column strips the alcohol from the beer as it flows downward and across each plate. The overhead vapors pass to the aldehyde column. Steam enters the base of this column. The main-product stream is withdrawn from the base at about 40° proof. Impurities tend to concentrate in the upper section. The "low boilers" or fusel-oil fraction is withdrawn three or four plates above the feed point and goes to the fusel-oil concentrating column. The "light boilers" or "heads," the fraction rich in aldehydes and esters, is withdrawn from the top of the column. These streams represent about 10 and 5% of the product stream, respectively.

The main stream of alcohol at low proof from the base of

the aldehyde column is pumped to the rectifying column. The lower section, comprising about twenty plates below the feed point, serves to strip alcohol from the low-proof mixture and the twenty to thirty-five plates in the upper section concentrate the alcohol to 190° to 192° proof and provide for further separation of a second fusel-oil fraction and a second heads draw from the condenser at the top of the column. Product alcohol is taken as a liquid draw from a plate near the top of the rectifying column and water is discharged from the base.

The fusel-oil draws are combined and fed to a fusel-oil column for further separation and concentration of fusel oil. A fraction containing a high concentration of fusel oil is drawn from the fusel-oil column and sent to a decanter; the water layer is returned to the stripping section of the oil column and the fusel oil to storage. A heads fraction is drawn from the top of this column, combined with the heads from the rectifying column, and fed back to the top of the aldehyde column.

Modern alcohol-distillation units are well instrumented and are efficient in recovering and purifying alcohol.

## Recovery of Grain Residues

The stillage discharged from the base of the beer still contains 6 to 8% of total solids. The use of approximately 20% of this residue in cooking and fermentation has been described. Thirty years ago, it was common practice to sell stillage by the barrel to farmers for wet feeding and to dispose of the excess in adjacent

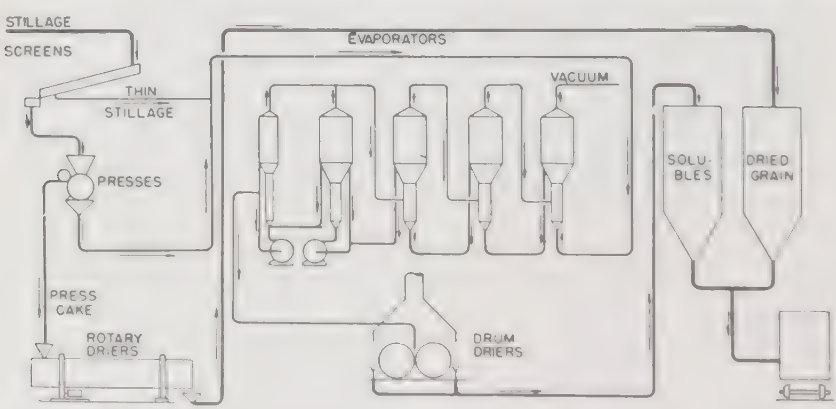



FIGURE 17. *Recovery of Distillers' By-Products* (Courtesy—Joseph E. Seagram & Sons, Inc., Louisville, Ky.)

streams. The enforcement of stream-pollution laws, the development of the mixed feed industry, and the rapid technological advances by the distilling industry in the past 15 years, have been responsible for total recovery of grain residues in dehydrated form by all modern plants. This has become a profitable operation of considerable economic importance to the distillery and to agriculture.

Figure 17 illustrates the operations in a typical distillery dryer house. Stillage is screened to remove a high percentage of the suspended solids; these are then dewatered in presses. The effluent from the screens and presses, or "thin stillage," is concentrated in multiple-effect evaporators to 30 to 35% solids. The resulting product is called evaporator sirup. The evaporator sirup may be dried on drum driers to produce distillers' dried solubles, as shown, or it may be combined with the press cake and dried in rotary driers to produce distillers' dark dried grains. If solubles are produced, then the press cake is dried separately to yield distillers' light dried grains. Boruff[12] has described a modified process in greater detail.

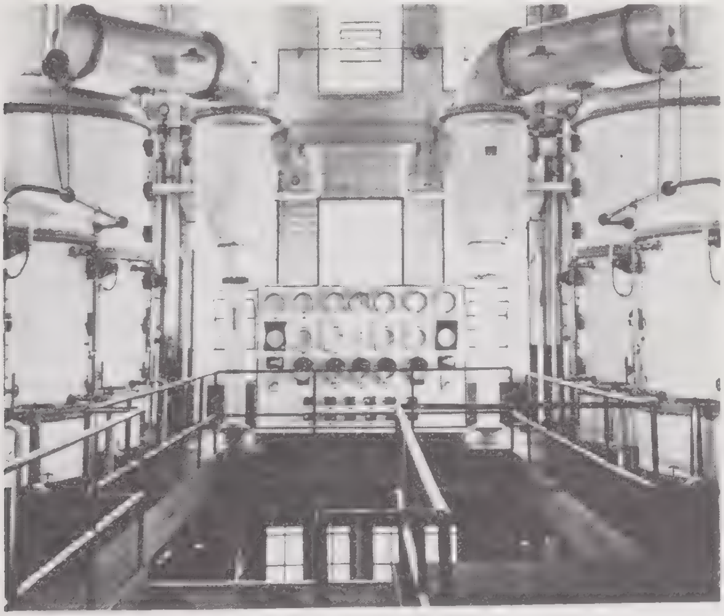


FIGURE 18. *Multiple-Effect Evaporator for Concentration of the Liquid Residue of Distillation (Stillage)* (Courtesy—Joseph E. Seagram & Sons, Inc., Lawrenceburg, Ind.)

Distillers' dried products are good sources of protein and the nutritional value of the original grain solids is enhanced by the yeast fermentation. A great deal of research on the use of these products in animal and poultry rations has been reported in the technical literature. The results of many recent studies have been published by the Distillers' Feed Research Council.[16,17,18]

In addition to the grain residues, another potential by-product from grain-alcohol plants is the carbon dioxide. This gas is produced during the fermentation and may be collected from closed fermentors, purified, and sold as liquid carbon dioxide or as "dry ice." Few grain distilleries recover the carbon dioxide. A discussion of this by-product is given in Chapter 3 on the alcoholic fermentation of molasses.

## ANALYTICAL METHODS

All phases of the manufacturing process are carefully controlled by analytical methods. Raw materials entering the plant are tested for quality. Processing steps, especially the actual fermentation, are controlled by analyses, and the final products are checked for purity.

### Raw Materials and Fermentation

Ground grain is tested as a routine for sieve analysis. Where efficiencies are to be determined, the ground grain is analyzed for moisture and starch contents. Routine bacteriological examinations are made on yeast inocula.

After filling a fermentor, "set analyses" are made on the fermentor contents. The initial volume of mash in the fermentor is measured and a sample of the contents taken to the laboratory for analysis. The analyses usually include measurements of Balling, pH, and titrable acidity. For these tests, a sample of the mash is strained to remove grain solids. By using a Balling hydrometer, the density of the mash is determined in degrees Balling. The Balling hydrometer scale expresses essentially the percentage of dissolved solids in the mash, 1° Balling representing 1% solids. Frequently the Balling reading is considered as the percentage of sugar; however, in addition to sugars, dextrins and other dissolved substances, all affect the density and thus the Balling reading. The pH of the strained mash is determined by means of a glass-electrode

pH meter and the titrable acidity by titrating a measured sample of the strained mash with standard alkali. Although custom varies in different plants, acidities are frequently expressed as milliliters of 0.1 *N* sodium hydroxide solution required to titrate 10 ml mash to the phenolphthalein end point.

During the fermentation, samples are taken periodically from each fermentor for analysis to obtain data similar to those shown in Figure 14. Typically, the temperature of the mash in the fermentor is recorded and alcohol content, Balling, pH, and titrable acidity determined on a sample of the fermenting mash. Alcohol is determined by distilling a measured volume of the mash and collecting a definite volume of distillate on which alcohol determinations are made by measuring specific gravity, or refractive index, or boiling point and referring the readings obtained to appropriate tables. Balling hydrometer readings, pH determination, and acidity titrations are run on strained samples of the mash. These combined determinations give an indication of the progress of the fermentation. If the fermentation is normal, the curves obtained for a fermentor will be similar to those shown in Figure 14. Contamination in a fermentor will be evidenced by high titrable acidity and low pH. Under these conditions, the fermentation will be slow and incomplete and the Balling readings high.

At the end of the fermentation, the final volume of the beer in the fermentor is measured and the final alcohol content, final Balling, final pH, and final titrable acidity are determined. From the final mash volume and data on grain charged to the fermentor and alcohol content of the beer, the yield is calculated.

## Products of Distillation

In the modern distillery, the quality of the products (both of the main product alcohol and of the by-products) is controlled by laboratory analyses. During distillation, samples of the stillage are tested for "slop loss," that is, for any alcohol not stripped from the beer but left in the stillage. The product alcohol is analyzed for alcohol concentration (proof), aldehydes, esters, organic acids, and fusel oil. Methods of the Association of Official Agricultural Chemists (A.O.A.C.)[4b] are in general use for these determinations although many laboratories use other methods of analysis. The grain residues are analyzed for moisture, protein, fat, ash, and

nitrogen-free extract by the usual methods of proximate feed analysis.

## FACTORS INFLUENCING ALCOHOL YIELD

The alcoholic fermentation of malt-converted grain mashes is particularly interesting, since two associated biological processes are conducted simultaneously in the presence of potentially competitive bacterial processes. The compromise conditions under which the two primary processes are conducted are not optimum for either and yet must be maintained to achieve the maximum alcohol yield. The two processes are the enzymic hydrolysis of the residual dextrins to fermentable sugars and the conversion of the sugars to alcohol by yeast fermentation. Both are operative during the main fermentation and the failure of either one will be reflected in reduced efficiency and low alcohol yields.

There are many factors that have a significant effect on alcohol yield and efficiency. Certain of these, particularly the factors that are integral with process modifications and procedures, have been discussed previously. The remainder may be classified as: (1) the physiological condition of the inoculum, (2) the environmental factors present during fermentation, and (3) the quality of the raw materials.

### Physiological Condition of the Inoculum

The physiological condition of the inoculum, the quantity and the cell population are of considerable importance. Fortunately, most yeasts are resistant to adverse environmental conditions and are adaptable to a wide range of conditions. Thus, the condition of the inoculum is by no means as critical as, for example, in the case of butyl alcohol fermentation. However, the realization of the maximum alcohol yield does require growth of the inoculum under optimal conditions.

Experience has shown that an actively growing yeast culture in the logarithmic growth phase and in an amount equivalent to 2% of the final fermentation volume provides sufficient yeast cells to initiate rapid growth and fermentation. The cell count varies with the yeast strain and medium. The procedure described earlier will result in a cell concentration of 150 million to 200 million per milliliter with most distillery yeast strains. The activity of the

inoculum may be arrested by holding at low temperatures for use several hours later. This procedure has no deleterious effect, although experience has shown that it is not desirable to use a culture that is more than 24 to 26 hours old, since older cultures may result in a yield reduction of 2 to 4%.

Until recently, it was widely believed in the industry that the primary criteria of a good yeast mash were the ability to support rapid yeast growth and to develop the normal population for the given strain. This hypothesis is open to question as recent reports offer evidence that a yeast mash may meet these criteria and yet lack necessary growth factors. Correction of the deficiency results in an increased efficiency in the subsequent fermentation. Scalf and coworkers[37,41] found this to be particularly true of aerobically grown inoculum. Their data indicate that a lack of these factors in the medium for inoculum may not affect yeast growth or sugar utilization in the fermentor appreciably, but has a marked effect on the efficiency of the conversion of sugar fermented to ethyl alcohol. Boyer and Underkofler[13] reported plant data showing that the use of mold bran in the yeast mash resulted in more vigorous yeast and increased the yield of alcohol. These results have been described in greater detail by Underkofler, Severson, and Goering.[44] Van Lanen, LeMense, Anellis, and Corman[50] have reported similar results with the addition of papain. The last two groups were working with yeast mashes ordinarily considered as bordering on the deficient, that is, containing 20 to 25% barley malt with 80 to 75% corn.

## Environmental Factors

It is necessary to discuss each of these factors separately and yet the interrelationship must not be subjugated and should always be kept in mind. As stated before, the alcoholic grain fermentation is a compromise. Barley malt not only provides amylase, but also contaminates the mash. High yields can only be attained by maintaining such conditions that the residual amylases will continue to function until the conversion of residual dextrins is complete, and the fermentable sugar so produced is converted to alcohol. The critical environmental factors are: (1) pH, (2) buffer capacity, (3) initial load of contaminants, (4) temperature, (5) mash concentration, (6) alcohol concentration, and (7) yeast nutrients.

pH

Grain mashes, without pH adjustment with acid or stillage, will enter the fermentor at pH 5.4 to 5.6. Stillage added during cooking will reduce the pH and if the amount of stillage is 20% of the final mash volume, then the pH of a corn-spirits mash will be approximately 4.9 to 5.0 at the fermentor.

The principal microbial contaminants in a distillery are lactic acid formers. The development of these microorganisms from a relatively low concentration, or at a low initial load, is repressed severely at pH values under 5.0. Above pH 5.0, their growth is rapid. Thus, it has been found most desirable to adjust the initial pH to 4.8 to 5.0 with either stillage or sulfuric acid. Rye mashes usually require acid in addition to 20% by volume of stillage due to a higher buffer capacity.

As soon as the pH of a fermentation falls below about 4.1, the amylases are inactivated. If this occurs prior to the complete conversion of the residual dextrins, low yields result. In the absence of acid-forming bacteria, the final pH will be 4.1 to 4.4, depending on the type of grain and the amount and type of stillage. The adjustment of the initial pH to 4.8 to 5.0 thus extends the period of residual dextrin conversion. This becomes of increasing value in obtaining high yields as the initial number of acid formers increases. Experimental evidence has been given by Willkie, Kolachov, and Stark.[52] It should be emphasized that pH adjustment is no panacea and failure to observe sanitary plant practices will still result in yield reductions of as much as 20% even with adjustment of the initial pH.

Attempts to salvage grossly contaminated fermentations by addition of lime, caustic, or soda ash invariably fail, since this accelerates the lactic fermentation and the rate of acid production at the expense of alcohol formation will actually exceed any practical rate of alkali addition. The end result is a still greater reduction in yield. This is a measure that has been tried occasionally in many plants.

The only successful procedure to date for pH control in the desired range is sanitary plant operation and reduction of the natural pH level of grain mashes with stillage and/or acid to 4.8 to 5.0 at the start of fermentation. Some distilleries prefer adjustment to as low as pH 4.5 and report successful results.

### Buffer Capacity

The buffer capacity of the mash is important. Grain mashes are generally well buffered from pH 5.0 to 6.0, poorly buffered from pH 4.4 to 5.0, and well buffered from pH 3.5 to 4.4. Stillage is of added value again, since it adds considerably to the buffer capacity of the mash between pH 3.5 and 4.4. This aids in stabilizing the pH above 4.1 for the maximum period and is one reason why stillage mashes give higher average yields than mashes from the grains without stillage.

### Initial Load of Contaminants

Good process sanitation is most important in the control of contamination and the realization of maximum alcohol yields. Due to the physiological conditions under which fermentation takes place, it is not necessary to resort to the drastic procedures used with fermentations such as those producing butyl alcohol or penicillin. Displacement of steam with sterile air, maintenance of positive pressure, double valving, and overlapping sterilization are not required. However, piping installation and equipment design should make provision for adequate steam sterilization, ease of cleaning, and the elimination of mash and condensate pockets. The practices and equipment for cleaning and sterilization procedures for distilleries are adequately described by Willkie and Prochaska.[53] Close adherence to the recognized principles is essential in holding the initial number of contaminants to a minimum.

It is recognized that in a production unit, it is desirable to operate the mashing and cooling systems for the maximum length of time between clean-ups. This has led to several attempts to establish the maximum permissible load of contaminants. Little information has been published since there is considerable variation, depending on the method of counting bacteria, plant design, and operating procedure. The Seagram group[23] has found that 10,000 bacteria per milliliter, as determined by the thioglycollate tube dilution method,[38] represents a level below which contamination is not a yield factor. Above these levels, lower alcohol yields may be anticipated, although they do not always occur.

### Temperature

Gray, Stark, and Kolachov[26] have reported that the optimum temperature for the growth of certain strains of distillers' yeast is 84° to 90°F as compared with an optimum fermentation temperature

of 100°F in the absence of alcohol, and 90°F at alcohol concentrations above 4.5% by volume. The maximum growth temperature of most yeasts under these conditions is about 90°F. It has been pointed out that the protracted fermentation period due to dextrin conversion makes it necessary to maintain the yeast population in good condition. Failure to control the temperature within the optimum-growth temperature range destroys yeast activity in a few hours and thus sharply reduces the alcohol yield. If the temperature exceeds 90°F for any appreciable length of time during the first 24 hours, then yield reductions of 10 to 15% may result. Furthermore, the high temperatures favor the growth of bacterial contaminants. The molasses fermentation (Chapter 3) may be conducted at higher temperatures than the grain fermentation, since the carbohydrate is present initially in a fermentable state and no enzymic saccharification is involved.

### Mash Concentration

The initial concentration of the mash governs both the final alcohol concentration and the heat release per unit volume. It is, therefore, necessary to employ a concentration that will neither generate more heat than can be dissipated without exceeding 90°F nor potentially result in an alcohol concentration in excess of the practical alcohol tolerance of the yeast strain. However, an unnecessarily dilute mash increases the steam consumption for distillation and by-product recovery and reduces plant capacity. Concentrations of 35 to 38 gal of mash per bushel of grain are customary, although concentrations as high as 30 gal of mash per bushel have also been used, usually with some alcohol-yield reduction. During World War II, yield was sometimes sacrificed in the interest of an over-all increase in plant capacity and output.

### Alcohol Concentration

The fermentation rate of yeast is greatly reduced in the presence of ethyl alcohol as previously discussed. It is apparent that a high mash concentration and resultant high final alcohol concentration will protract the fermentation period, possibly to a dangerous degree, since the added time favors development of contaminants.

### Yeast Nutrients

Small-grain mashes (wheat, rye, barley malt) are relatively rich in nutrients, while corn mash with 8 to 10% of barley malt is

marginal in nutrient level. None of these require supplementing with inorganic nitrogen, as this does not result in yield increases. Most of the nutrients in corn mashes are contained in the distillers' barley malt. Stillage has appreciable nutrient value and is added for this reason and also for its buffering value and aid in adjusting the pH.

If the content of distillers' barley malt in a mash is reduced below 8%, then sufficient amylase may be present but inadequate nutrients. It has been found that 6% distillers' barley malt in a corn mash may result in a 2 to 5% reduction in yield. This has been overcome by the addition of 0.5 to 1.0% of malt sprouts.[36] Van Lanen, LeMense, Anellis and Corman[50] have investigated the relationship between malt requirements, proteolytic enzymes, yeast nutrients and alcohol yield. They concluded that corn mash containing 8 to 10% of malt is suboptimal with respect to amino nitrogen and were successful in accelerating the fermentation rate with proteolytic enzymes or with protein hydrolyzates. The supplements had no beneficial effect on yield except when the malt level was reduced to about 4%. Part of their data on supplements added to mashes with reduced levels of malt appears in Table 9. It will be noted that the use of papain and low levels of casein hydrolyzate or urea added to 4% malt mashes increased the yield to almost the same level as the 10% malt mash. Efficiencies were not as high since the 4% malt mash had a higher average starch content.

Data in the same table also illustrate the customary reduction in yield that results from high levels of added nitrogen, particularly with urea or ammonia nitrogen. As has been suggested,[14,50] this is probably due to ammonia being fixed in carbonyl groups such as pyruvic acid and shunting a part of the carbohydrate out of the fermentation cycle. Adams, Woods, and Stark[4] have reported similar data from attempts to increase the protein content of by-product feeds by the addition of urea to regular distillery mashes.

## Quality of Raw Materials

### Starch Source

The alcohol yield is in direct proportion to the starch content of the grain bill, all other factors being equal. Damaged grain, as reflected by moldiness, broken kernels, etc., will ferment as well as

Table 9. EFFECT OF CASEIN HYDROLYZATE, UREA, AND STILLAGE ON THE FERMENTATION OF CORN MASHES SACCHARIFIED WITH A SUBOPTIMAL LEVEL OF MALT

| Conversion malt, % | Nitrogen supplement per 100 ml of mash | *Weight Loss in Grams* 18 hours | 42 hours | 68 hours | Final pH | Yield of alcohol proof gal per bu |
|---|---|---|---|---|---|---|
| 10 | None | 7.5 | 15.5 | 16.7 | 4.3 | 5.17 |
| 4 | None | 5.6 | 13.6 | 15.9 | 4.1 | 4.76 |
| 4 | 20 ml stillage | 7.5 | 13.8 | 16.2 | 4.2 | 4.92 |
| 4 | 10 mg papain | 11.4 | 15.3 | 16.8 | 4.5 | 4.10 |
| | Casein hydrolyzate, amino-nitrogen equivalents (mg) | | | | | |
| 4 | 2 | 7.1 | 14.4 | 16.2 | 4.3 | 5.04 |
| 4 | 6 | 9.2 | 14.6 | 16.3 | 4.4 | 5.13 |
| 4 | 12 | 10.7 | 14.5 | 16.4 | 4.5 | 5.14 |
| 4 | 24 | 12.2 | 15.4 | 16.5 | 4.6 | 5.20 |
| 4 | 40 | 12.7 | 14.7 | 16.1 | 4.6 | 5.05 |
| | Urea-nitrogen equivalents (mg) | | | | | |
| 4 | 2 | 6.7 | 12.8 | 15.8 | 4.3 | 4.83 |
| 4 | 6 | 8.4 | 14.3 | 16.0 | 4.3 | 5.03 |
| 4 | 12 | 9.3 | 14.2 | 16.0 | 4.4 | 4.89 |
| 4 | 24 | 11.6 | 14.3 | 15.9 | 4.4 | 4.86 |
| 4 | 40 | 12.3 | 14.1 | 15.7 | 4.5 | 4.68 |

Source: Van Lanen, Le Mense, Anellis, and Corman.[50]

grain of better quality unless the degree of damage is very high. However, this condition may result in increased grain losses on cleaning and may have an adverse effect on the quality of alcohol produced and is, therefore, undesirable. The bacterial content of the grains is not important when the mash is cooked at 212°F or above. Adams, Stark and Kolachov[3] have shown that kiln drying of corn at temperatures in excess of 200°F will result in 2 to 3% decrease in alcohol yield, apparently due to starch degradation. A high percentage of new corn is kiln dried during fall and early winter months.

### Barley Malt

The enzyme content of barley malt has a pronounced effect on the alcohol yield. At one time it was believed that $\beta$-amylases, or the saccharifying enzymes, were of primary importance and the $\alpha$-amylases, or liquifying enzymes, were secondary. On this basis, the Lintner value which reflects $\beta$-amylase content was believed to be

the only necessary specification. Malts with Lintner values over 170° dry basis were believed to be of uniformly high quality. Some distillers have made the Lasche test[15] the basis of their specifications. Recognition of the importance of the $\alpha$-glucosidase systems in residual or limit dextrin conversion and advances in the chemistry of enzymic starch hydrolysis have led to further investigations of the relationship between alcohol yield and the several enzyme systems present in barley malts. This has been stimulated by the economic desirability of using minimum concentrations of malt.

Thorne, Emerson, Olson, and Peterson,[43] in a study on malt evaluation, found significant correlations between both $\alpha$- and $\beta$-amylase values and alcohol yields from wheat mashes converted with 2% malt and fermented for 72 hours, and between $\alpha$-amylase only and alcohol yield at a 5% malt level with 36-hour fermentations. It was their conclusion that data on the amylase contents of malts are informative, but undetermined factors play an important role and malt can be evaluated only by fermentation tests. Whitehouse, Delehanty, Scalf, and Smith,[51] employing a modification of Thorne's reduced malt level technique that compensates in part for nutrient factors, have obtained good correlation between $\alpha$-amylase values and alcohol yield. Their data indicate a range in yield from 9.3 to 10.5 proof gallons per 100 lb of corn (dry basis), depending on the $\alpha$-amylase content of the barley malts that were used. The higher $\alpha$-amylase levels produced the higher alcohol yields.

The conversion of limit dextrins is a phenomenon about which surprisingly little is known, considering its technical and economic importance. Pigman[32] has presented data in support of the belief that the hydrolysis of starch by malt amylases reaches an equilibrium in the absence of yeast fermentation. During fermentation, maltose is removed and this allows the reaction to proceed. Back, Stark, and Scalf[6] have presented data indicating that limit dextrins are converted by a third enzyme system. Witt and Ohle,[54,55,56] using a different approach to the problem, have concluded that $\beta$-amylase may be of greater importance than $\alpha$-amylase, but also state that a third, relatively heat labile system is essential for maximum alcohol yield. They are obviously not in disagreement with Pigman, but presumably have gone further into the mechanism of the over-all reaction. There are sufficient data to substantiate the belief that in addition to the $\alpha$- and $\beta$-amylases, at least one enzyme system, is

essential for complete conversion and maximum alcohol yields.

A cooperative research program conducted by several laboratories under the coordination of the Malt Research Institute Laboratories at Madison, Wisconsin, has been in progress for several years. The results of this program are expected to clarify many of the interrelationships between barley malt and alcohol yield.

Barley malt is the primary source of bacterial contaminants in mash. Thus, the type of bacteria and the total numbers entering the process are important yield factors. It was commonly believed that the bacterial content of barley malt should be held to a minimum. This was made the basis of a patent[5] for the formaldehyde treatment of barley malt. Adams, Stark, and Kolachov[2] have shown that the pasteurization effect of the conversion temperature of 145°F for as short an exposure time as 1 minute reduces the number of contaminants below the critical level, regardless of the initial bacterial content of the malt. However, this is not always true, since infrequently contamination resulting in yield reductions of 10 to 15%, at times when all other factors could be eliminated, has been traced to bacterial contaminants introduced with the malt. The type of bacteria responsible has been found to be a very fast-growing, acid-forming short rod (almost coccoidal), that is evidently thermoduric and able to initiate growth at pH levels below 4.8. Fortunately this contaminant is found very infrequently.

A better understanding of the interrelationship of the factors that influence the yield of alcohol from grain-mash fermentations may be realized by a study of the data previously presented in Figure 14. The changes in pH, titrable acidity, sugar concentration (Balling), alcohol concentration, cell population and temperature during normal fermentation of a corn-spirits mash are illustrated graphically. This fermentation was conducted with temperature control in the range of 80-89°F and with some agitation due to recirculation of the mash through external heat exchangers. Thus fermentation was complete in 37 hours after inoculation. The alcohol yield was very good as would be expected from the final conditions of low acid rise, pH above 4.0, and negative Balling. It will be observed that 10% barley malt was used and that the initial pH was adjusted with sulfuric acid, since no stillage was used. The curves would be similar in the absence of temperature control, except for a lower setting temperature (66° to 72°F), and the fermentation period would be extended to 56 to 70 hours.

It would perhaps be of still greater value if Figure 14 might be accompanied by graphs illustrative of abnormal conditions. However, the presentation of a normal set of data will aid those who may have occasion to interpret and analyze data from alcoholic fermentations of grain mashes.

## RECENT IMPROVEMENTS IN THE ALCOHOLIC GRAIN-FERMENTATION PROCESS

There are other new developments in methods of yeast cultivation, starch conversion, and fermentation not in commercial use. Those that will be discussed in this section have been studied on pilot-plant or semi-commercial scale and it is anticipated that the adoption of some of these improvements may materially alter the commercial alcoholic grain-fermentation process.

### Yeast-Culture Preparation

A method for the continuous aerobic production of pure culture distillers' yeast,[46] has been described. A sterile wort is used as a medium and, due to vigorous aeration, the cell count is approximately three times the maximum under anaerobic conditions. The cycle during the continuous phase is 4 hours and all operations are carried out under aseptic conditions. Thus, the continuous aerobic process requires much less equipment. Furthermore, the elimination of contamination is a step toward aseptic distillery operation, which, of course, can be achieved with proper anaerobic technique also.

### Starch Conversion

The less satisfactory aspects of the conventional use of barley malt for starch conversion have been discussed. The desire to avoid the problems that accompany incomplete conversion of starch and to eliminate barley malt as a source of contamination has motivated a great deal of research on the alternate use of either mineral acids or other enzyme systems that have higher activity and are free from contaminants.

#### Acid Hydrolysis

Corn sugar (dextrose) has been produced for some years by the acid hydrolysis of starch. The development of a process for con-

tinuous acid saccharification of grain mashes for fermentation purposes has been described by Unger and Grubb.[45] The alcohol yields obtained from the fermentation of mashes saccharified by this process are invariably lower than from malt-converted mashes unless organic nutrient materials, such as mold bran or *Aspergillus oryzae* produced in submerged culture, are added.[35]

### Fungal Amylases

The use of mold preparations, obtained by both surface cultivation on bran and in submerged culture, has been well known in the Orient and in Europe. However, recent studies, stimulated in part by the shortage of malt during World War II, have resulted in improvements in both processes. These are described in some detail in Chapter 3 of Volume II.

**Mold Bran.** Hao, Fulmer, and Underkofler[27] investigated twenty-seven mold strains and selected three strains of *Aspergillus oryzae* which produced mold bran that resulted in good alcohol yields. The results of plant-scale trials of this process have been summarized by Underkofler, Severson, and Goering[44] and some of their data are presented in Table 10. They reported that average yields of alcohol in plant fermentations were 2% higher with 4% mold bran as the saccharification agent, as compared with yields from malt-saccharified mashes.

Table 10. RESULTS OF PLANT-SCALE TESTS WITH MOLD BRAN

| Number of fermentors | Saccharifying agent in fermentor mashes % | Saccharifying agent in yeast-culture mashes % | *Average Alcohol Yield per Standard bu* proof gal | wine gal 190° proof |
|---|---|---|---|---|
| 299 | 10 malt | 22 malt | 4.77 | 2.51 |
| 847 | 9–10 malt | 8.6 malt + 4.3 mold bran | 5.17 | 2.72 |
| 6 | 4 mold bran | 8.6 malt + 4.3 mold bran | 5.24 | 2.76 |
| 12 | 9–10 malt | 8.6 malt + 4.3 mold bran | 5.15 | 2.71 |
| 7 | 3.9–6.2 malt + 2.2–0.9 mold bran | 8.6 malt + 4.3 mold bran | 5.26 | 2.77 |
| 12 | 9–10 malt | 8.6 malt + 4.3 mold bran | 5.23 | 2.75 |

Source: Underkofler, Severson, and Goering, *Ind. Eng. Chem.*, **38**, 980 (1946) (Reprinted by permission).

**Submerged-Culture Amylases.** The previously mentioned, Amylo process was the original process of this type. Its disadvantages were growth in the main fermentor mash, increased steam consumption, and relatively high plant investment, as compared with recently developed processes which utilize grain stillage as the growth medium and other fungi as the cultures.

Erb and Hildebrandt[19] have obtained improved yields of alcohol from granular wheat flour when malt was partially replaced with enzymic material obtained by cultivating *Rhizopus* strains in a mash-stillage medium. Balankura, Stewart, Scalf, and Smith[8] reported on the use of *Aspergillus oryzae* grown on stillage and Van Lanen and Le Mense,[49] on a process using a strain of *Aspergillus niger*. The latter organism has been found to be superior and further work on the process, both in the laboratory and the pilot plant, has been reported.[1,29] Selected data from these two papers appear in Table 11.

Table 11. SUBMERGED MOLD CULTURE[a] AS A STARCH-CONVERSION AGENT IN THE ALCOHOLIC FERMENTATION OF GRAIN

| *Conversion Agent* Type | Amount | Alcohol yield proof gal per bu | Plant efficiency % |
|---|---|---|---|
| | | dry basis[b] | |
| Malt | 8% by weight of grain | 5.95 | 91.3 |
| *A. niger* | 10% by volume | 6.27 | 94.0 |
| *A. niger* | 10% by volume | 6.20 | 92.9 |
| *A. niger* | 10% by volume | 6.16 | 92.3 |
| | | as received basis[c] | |
| *A. niger* | 10% by volume | 5.03 | |
| *A. niger* | 10% by volume | 5.04 | |
| *A. niger* | 10% by volume | 5.20 | |
| *A. niger* | 10% by volume | 5.00 | |
| *A. niger* | 10% by volume | 4.82 | |
| *A. niger* | 10% by volume | 5.19 | |

[a] *Aspergillus niger*, NRRL 337.
[b] Data of Adams, Balankura, Andreasen, and Stark.[1]
[c] Data of Le Mense, Sohns, Corman, Blom, Van Lanen, and Langlykke.[29]

The *A. niger* process makes possible aseptic operation by the complete elimination of barley malt, is economically attractive as compared with the use of barley malt, and results in 4 to 6% higher alcohol yields. It is, therefore, quite possible that the widespread industrial application of this process will develop in the near future.

The quality of beverage-alcohol distillates produced from commercial trials was found to be equal or superior to those obtained from malt-converted mashes.

Extensive commercial-scale experiments with submerged culture of *A. niger* have recently been reported.[47a] From these experiments, it was estimated that a plant using 12,000 bu of grain a day could save more than $1,000 a day by shifting to the new process. Conversion to the fungal process was relatively simple and the process had no adverse effects on the quality of the alcohol or by-product feeds.

### Continuous Fermentation of Grain Mashes

Research work has been in progress for several years in an effort to develop a continuous, fast fermentation process that would eliminate the necessity for the large number of fermentors now required. This has been made difficult by the slow conversion of residual dextrins and the contamination introduced with barley malt. The elimination of these two problems has been responsible for much of the work on acid and fungal amylase saccharification of starch.

Ruf, Stark, Smith and Allen[35] have described pilot-plant trials of a process for the continuous fermentation of acid-saccharified mashes. They have claimed the development of an economic process. However, the regulations of the Alcohol Tax Unit in the United States limit the application of acid-saccharification processes in beverage-alcohol production in this country. Thus, it is unlikely that this process will gain commercial acceptance for some time.

There are many advantages inherent in a continuous fermentation process and it is probable that the continuous alcoholic grain fermentation will become general on solution of the problems of conversion rate and contamination from both the technical and governmental aspects.

## ALCOHOLIC FERMENTATION OF OTHER STARCHY SUBSTRATES

Of the many possible starchy raw materials, the various grains are the principal substances employed in the fermentation industries of the United States. However, during recent years, potatoes have also been used on an industrial scale for alcohol fermentation in

this country. They have long been a principal raw material for alcohol manufacture in Europe.[20] In tropical countries, cassava, said to be the cheapest known source of starch, offers much promise and has found industrial application as a fermentation substrate.[42a]

The procedures for the production of alcohol from grains, previously outlined in this chapter, are generally applicable to the processing of other starchy substrates, such as potatoes, sweet potatoes, and cassava. Such tuber and root crops ordinarily have a high moisture content and spoil easily. Therefore, the processing of these crops must take place promptly after harvesting or they may be dehydrated for storage for later processing. However, dehydration is ordinarily too costly for extensive use.

Dehydrated tuber and root crops may be ground and processed in a similar manner to grains. The fresh crops require some variation in cooking procedures, due to their high moisture content. Ordinarily, batch-pressure cooking is used and the Henze converter has found much favor in Europe for cooking whole potatoes.[20] For the continuous cooking process, the fresh roots or tubers must be disintegrated in suitable grinders, with a minimum volume of added water, before cooking.

Experience has shown that fungal amylase preparations are much superior to malt for saccharification of potatoes,[10a] sweet potatoes,[43a,27a,27b] and cassava.[9a,42a] Mashes of these substrates converted with fungal amylase are thinner and alcohol yields are better than with malt-saccharified mashes. For example, Teixeira, Andreasen and Kolachov[42a] reported yields, per 100 lb of dehydrated cassava meal (as received basis), of 11.6 proof gallons with submerged culture of *A. niger* as compared with 9.2 proof gallons with barley malt.

## BIBLIOGRAPHY

1a. Adams, S. L., Jos. E. Seagram & Sons., Inc., Louisville, Ky. Personal communication.

1. Adams, S. L., B. Balankura, A. A. Andreasen, and W. H. Stark, *Ind. Eng. Chem.*, **39**, 1615 (1947).
2. Adams, S. L., W. H. Stark, and P. Kolachov, *J. Bact.*, **42**, 292 (1941).
3. Adams, S. L., W. H. Stark, and P. Kolachov, *Cereal Chem.*, **20**, 260 (1943).

4. Adams, S. L., J. K. Woods, and W. H. Stark, *Abstracts Proc. Soc. Am. Bact.*, p. 40 (1947).
4a. Arnold, L. K., and L. A. Kremer, *Corn as a Raw Material for Ethyl Alcohol, Iowa Eng. Exp. Sta., Bull.* **167** (1950).
4b. Association of Official Agricultural Chemists, *Official and Tentative Methods of Analysis,* 6th ed, Washington, D. C., Association of Official Agricultural Chemists, 1950.
5. Atwood, H. G., U. S. Patent 2,329,509 (1943).
6. Back, T. M., W. H. Stark, and R. E. Scalf, *Anal. Chem.*, **20**, 56 (1948).
7. Back, T. M., W. H. Stark, and C. C. Vernon, *Ind. Eng. Chem.*, **40**, 80 (1948).
8. Balankura, B., F. D. Stewart, R. E. Scalf, and L. A. Smith, *J. Bact.*, **51**, 594 (1946).
9. Baldwin, E., *Dynamic Aspects of Biochemistry,* New York, The Macmillan Co., 1947.
9a. Banzon, J., E. I. Fulmer, and L. A. Underkofler, *Iowa State Coll. J. Sci.*, **23**, 219 (1949).
10. Barron, H., *Distillation of Alcohol,* Louisville, Ky., Div. of Edu., Jos. E. Seagram & Sons, 1944.
10a. Beresford, H., and L. M. Christensen, *Idaho Agr. Expt. Sta., Bull.* **241** (1941).
11. Blankmeyer, H. C., and W. H. Stark, U. S. Patent 2,375,189 (1945).
12. Boruff, C. S., *Ind. Eng. Chem.*, **39**, 602 (1947).
13. Boyer, J. W., and L. A. Underkofler, *Chem. & Met. Eng.*, **52**, No. 12, 110 (1945).
14. Brockmann, M. C., and T. J. B. Stier, *J. Cellular Comp. Physiol.*, **29**, 159 (1947).
15. de Becze, G., *Am. Wine & Liquor J.*, **8**, No. 5, 29 (1941).
16. Distillers' Feed Research Council, *Proceedings,* Second Annual Conference, 1946.
17. Distillers' Feed Research Council, *Proceedings,* Third Annual Conference, 1947.
18. Distillers' Feed Research Council, *Proceedings,* Fourth Annual Conference, 1948.
19. Erb, N. M., and F. M. Hildebrandt, *Ind. Eng. Chem.*, **38**, 792 (1946).
20. Foth, G., *Handbuch der Spiritusfabrikation,* p. 351-357, Berlin, Paul Parey, 1929.
21. Foth, G., *Die Praxis des Brennereibetriebes auf wissenschaftlicher Grundlage,* p. 114-115, Berlin, Paul Parey, 1935.

22. Gallagher, F. H., H. R. Bilford, W. H. Stark, and P. Kolachov, *Ind. Eng. Chem.*, **34**, 1395 (1942).
23. Gallagher, F. H., and W. H. Stark, Unpublished data, Joseph E. Seagram & Sons (1941).
24. Gallagher, F. H., W. H. Stark, and P. Kolachov, *J. Bact.*, **41**, 91 (1941).
25. Gray, W. D., *J. Bact.*, **42**, 561 (1941).
26. Gray, W. D., W. H. Stark, and P. Kolachov, *J. Bact.*, **43**, 270 (1942).
27. Hao, L. C., E. I. Fulmer, and L. A. Underkofler, *Ind. Eng. Chem.*, **35**, 814 (1943).
27a. Hao, L. C., and W. H. Stark, 109th Meeting, Am. Chem. Soc. (1946).
27b. Jump, J. A., A. I. Zarow, and W. H. Stark, *Ind. Eng. Chem.*, **36**, 1138 (1944).
28. Kolachov, P., *Trans. Am. Assoc. Cereal Chemists*, **2**, 14 (1944).
29. Le Mense, E. H., V. E. Sohns, J. Corman, R. H. Blom, J. M. Van Lanen, and A. F. Langlykke, *Ind. Eng. Chem.*, **41**, 100 (1949).
30. Owen, W. L., *Ind. Eng. Chem.*, **25**, 87 (1933).
31. Packowski, G. W., S. L. Adams, and W. H. Stark, National Farm Chemurgic Council, *Chemurgic Papers*, Series 4, No. 593 (1947).
31a. Pfeifer, V. F., and C. Vojnovich, *Ind. Eng. Chem.*, **44**, 1940 (1952).
32. Pigman, W. W., *J. Res. Nat. Bur. Standards*, **33**, 105 (1944).
33. Porter, J. R., *Bacterial Chemistry and Physiology*, New York, John Wiley, 1944.
34. Rodgers, C. H., *Chem. Eng.*, **55**, No. 10, 122 (1948).
35. Ruf, E. W., W. H. Stark, L. A. Smith, and E. E. Allen, *Ind. Eng. Chem.*, **40**, 1154 (1948).
36. Scalf, R. E., Unpublished data, Joseph E. Seagram & Sons, (1941).
37. Scalf, R. E., and W. H. Stark, Unpublished data, Joseph E. Seagram & Sons, (1941).
38. Seagram, Joseph E., & Sons, Inc., *Control Laboratory Procedures*, Louisville, Ky., 1944.
39. Stark, W. H., S. L. Adams, R. E. Scalf and P. Kolachov, *Ind. Eng. Chem., Anal. Ed.*, **15**, 443 (1943).
40. Stark, W. H., P. Kolachov and H. F. Willkie, *Ind. Eng. Chem.*, **35**, 133 (1943).
41. Stark, W. H., R. E. Scalf, P. Kolachov, and H. F. Willkie, *Am. Soc. Brewing Chemists, Proc.*, **1941**, 49.

42. Stephenson, M., *Bacterial Metabolism,* 3rd ed., New York, Longmans Green, 1949.
42a. Teixeira, C., A. A. Andreasen and P. Kolachov, *Ind. Eng. Chem.,* **42**, 1781 (1950).
43. Thorne, C. B., R. L. Emerson, W. J. Olson, and W. H. Peterson, *Ind. Eng. Chem.,* **37**, 1142 (1945).
43a. Underkofler, L. A., Unpublished information.
44. Underkofler, L. A., G. M. Severson and K. J. Goering, *Ind. Eng. Chem.,* **38**, 980 (1946).
45. Unger, E. D., and H. W. Grubb, 107th Meeting, Am. Chem. Soc. (1944).
46. Unger, E. D., W. H. Stark, R. E. Scalf and P. J. Kolachov, *Ind. Eng. Chem.,* **34**, 1402 (1942).
47. Unger, E. D., H. F. Willkie, and H. C. Blankmeyer, *Trans. Am. Inst. Chem. Eng.,* **40**, 421 (1940).
47a. U. S. Dept. Agriculture, "Methods and Costs of Producing Alcohol from Grain by the Fungal Amylase Process on a Commercial Scale," *Tech. Bull. No. 1024* (1950).
48. U. S. Dept. Treasury, Bur. Industrial Alcohol, *Regulations 3,* "Relative to the Production, Tax Payment, etc., of Industrial Alcohol and to the Manufacture, Sale, and Use of Denatured Alcohol," Washington, D. C., 1942.
49. Van Lanen, J. M., and E. H. Le Mense, *J. Bact.,* **51**, 595 (1946).
50. Van Lanen, J. M., E. H. Le Mense, A. Anellis and J. Corman, *Cereal Chem.,* **25**, 325 (1948).
50a. Werkman, C. H., and P. W. Wilson, *Bacterial Physiology,* New York, Academic Press, 1951.
51. Whitehouse, K., M. E. Delehanty, R. E. Scalf and L. A. Smith, Paper presented at Ky. Acad. Sci., April, 1946.
52. Willkie, H. F., P. Kolachov, and W. H. Stark, *Cereal Chem.,* **17**, 760 (1940).
53. Willkie, H. F., and J. A. Prochaska, *Fundamentals of Distillery Practice,* Louisville, Ky., Jos. E. Seagram & Sons, 1943.
54. Witt, P. R., and R. L. Ohle, *Am. Soc. Brewing Chemists, Proc.,* **1947**, 45.
55. Witt, P. R., and R. L. Ohle, *Am. Soc. Brewing Chemists, Proc.,* **1947**, 50.
56. Witt, P. R., and R. L. Ohle, *Am. Soc. Brewing Chemists, Proc.,* **1947**, 55.

CHAPTER 3

# ALCOHOLIC FERMENTATION OF MOLASSES

*H. M. Hodge and F. M. Hildebrandt*

Large-scale production of industrial ethyl alcohol in the United States began soon after the industrial alcohol act of 1906, when alcohol used for industrial purposes (as denatured alcohol) was made tax exempt. Since that time, several billion gallons of industrial alcohol have been made from molasses, the most economical source of sugar for the fermentation industries for many years.

The various raw materials for alcohol production were mentioned in Chapter 2. Tousley[50] gives a summary of amounts of alcohol produced and lists important uses over a period of years up to 1941. The normal production and use of industrial alcohol, exclusive of beverage spirits, in the years preceding World War II was in the neighborhood of 100 million gallons per year. The total production of ethyl alcohol in the United States from several raw materials in the most recent years for which figures are available is shown in Table 12.

The technology of the yeast fermentation of molasses is simple and well worked out. Essentially, the process involves merely dilution of the molasses, inoculation with yeast, fermentation, and distillation. Problems of processing and control in the alcoholic

TABLE 12. PRODUCTION OF ETHYL ALCOHOL BY TYPES OF RAW MATERIAL

| Raw material | Proof gallons × $10^3$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1939 | 1941 | 1942 | 1943 | 1944 | 1945 | 1946 | 1947 |
| Grain[a] | 15,540 | 17,532 | 38,578 | 107,857 | 206,253 | 281,695 | 104,963 | 39,742 |
| Molasses[a] | 135,834 | 210,427 | 289,396 | 159,190 | 207,523 | 190,201 | 87,118 | 54,159 |
| Ethyl sulfate | 47,946 | 69,903 | 90,615 | 96,739 | 113,734 | 111,679 | 127,508 | 133,306 |
| Unfinished spirits and redistillation products | — | — | 5,098 | 81,454 | 45,246 | 32,920 | 23,692 | 49,546 |
| Sulfite liquors | — | — | — | — | — | 1,018 | 2,837 | 4,380 |
| Pineapple juice | 151 | 227 | 205 | 254[a] | 438 | 241 | 250 | 220 |
| Whey | — | — | — | — | 136 | 155 | 136 | 262 |
| Crude alcohols mixture | | | | | | | | |
| Cellulose pulp and chemical mixtures | 310[b] | 576 | 814 | 1,198 | 1,632 | 1,731 | 1,954 | 2,422 |
| Potatoes | — | — | — | — | 224 | — | 3,772 | 12,861 |
| Other mixtures (grain, molasses, whey, pineapple juice, etc.) | 1,218 | 180 | 97 | 1,095 | 61,386 | 63,791 | 1,296 | 1,983 |
| TOTAL ALCOHOL[c] | 201,018 | 298,845 | 424,804 | 447,787 | 636,575 | 683,432 | 353,524 | 298,881 |

[a] Additional amounts used in combination with other materials included under "other mixtures."

[b] Cellulose pulp included in "other mixtures."

[c] Gross production; includes products used in redistillation.

Source: Bureau of Internal Revenue reports.[3]

molasses fermentation are much simpler than in the alcoholic fermentation of grains. In recent years, most attention has been given in the ethyl molasses fermentation industry to: (1) methods of obtaining higher yields, (2) continuous fermentations, and (3) by-product utilization and disposal.

## RAW MATERIALS

### Blackstrap Molasses

Blackstrap molasses, used in ethyl alcohol fermentation, is a by-product of the sugar industry, obtained after the sucrose has been crystallized and centrifuged from defecated, evaporated cane juice. The process of evaporation and crystallization is usually repeated three times until the invert sugar, nonsugar organic constituents, and high viscosity of the molasses will permit no further crystallization of the sucrose. The residue is known as final or blackstrap molasses. Blackstrap molasses is, therefore, a rather crude, complex mixture, containing sucrose, invert sugar, salts, and all of the alkali-soluble nonsugar ingredients normally present in the defecated cane juice, as well as those formed during the process of sugar manufacture.

In addition to sucrose, glucose, fructose and raffinose, which are fermentable, blackstrap molasses also contains reducing substances not fermentable by yeast. These copper-reducing, nonfermentable compounds are mainly caramels, free from nitrogen, produced by the heating necessary during sugar manufacture, and melanoidins, containing nitrogen and derived from condensation products of sugars and amino compounds.[31,51]

Sattler and Zerban[43,44,45] have reported on a study of the unfermentable reducing substances in molasses. This is of considerable interest to the fermentation industry since a more complete knowledge of the unfermentables in this important raw material may indicate how their amount can be decreased. The nonfermentable reducing content of molasses may be as high as 17% in blackstrap and as low as 5% in high-test molasses. Sattler and Zerban[45] reported that about 10% of the reducing power of the nonfermentable substances in cane molasses was due to volatile ingredients, such as hydroxymethylfurfural, acetoin, formic acid and levulinic acid, which were decomposition products of the sugars.

It is obvious that reduction of the nonfermentable substances would be of great economic importance. The work of Sattler and Zerban was carried out with this particular application in mind. They showed that the unfermentable substances were formed largely at the expense of fructose which is especially likely to undergo transformation to reducing compounds not available to yeast. If, for instance, a concentrated solution of fructose is boiled gently for a period of 16 hours under reflux, a considerable fraction of the sugar undergoes dehydration and condensation reactions that yield nonfermentable reducing substances. Sattler and Zerban showed that the resulting substance, formerly called "glutose," is a mixture of 1,3-fructopyranose anhydride, its nonreducing dimer, and dark-colored fructose caramel. Another type of reaction resulting in unfermentable substances in condensation between both glucose and fructose and the amino acids in cane juice. All of these reactions are accelerated by heat, and the authors call attention to the fact that both the high temperature used in processing cane juice in sugar mills and the subsequent storage of molasses under the hot tropical sun increase the amount of nonfermentable substances. Although some injury due to heat is unavoidable in the process of sugar production, there is no question that the loss of fermentability in molasses could be decreased by cooling the final product before storage and by avoiding very high sugar concentrations in such products as high-test molasses. Sattler and Zerban found sugars could be regenerated from some of the condensation products by mild acid hydrolysis and suggest this might increase the alcohol yield in distilleries operating on molasses. Erb and Zerban[15] outlined methods for calculating the probable alcohol yield from normal molasses and for the detection of abnormal molasses.

The bulk of the blackstrap molasses imported into the United States comes from Cuba, with lesser amounts originating in Puerto Rico and the Dominican Republic. On the west coast of this country, considerable amounts of molasses from Hawaii are fermented. Before World War II, some Javan molasses was also imported. Cuba normally produces about 175 million gallons of blackstrap per year. Typical blackstrap molasses shows a composition within the range: solids 83 to 85%, sucrose 30 to 40%, invert sugar 12 to 18%, ash 7 to 10%, and organic nonsugars 20 to 25%. About 90% of the total sugars is fermentable by yeast.

**High-test Molasses**

The so-called high-test or invert molasses, used in large quantities prior to 1942 and again available in 1953, is obtained by direct evaporation of defecated cane juice, no attempt being made to obtain crystalline sucrose. In the manufacture of high-test molasses, the sugar is partially inverted with mineral acids or by means of yeast having a high invertase content, this inversion serving to avoid subsequent crystallization of sucrose during storage and transport.

High-test molasses had its origin during the depression years (1932-1933) when a surplus of sugar was being produced and the demand for molasses sugar was greater than that produced by blackstrap alone. In 1941, Cuba produced 331 million gallons of high-test molasses. Analysis of high-test molasses shows a composition within the range: solids 80 to 85%, sucrose 15 to 35%, invert sugar 60 to 40%, ash 2 to 4%, and organic nonsugars 4 to 8%. About 95% of the sugars in high-test molasses is fermentable by yeast.

## FERMENTATION MECHANISM

The fermentable carbohydrates in molasses are sugars, principally sucrose and invert sugar. These are directly fermentable by yeasts. The mechanism by which sugars are converted into alcohol by yeast has been given in Chapter 2.

## CULTURES, THEIR MAINTENANCE AND DEVELOPMENT TO PLANT STAGE

The prerequisites of a good yeast culture for alcohol production from molasses are: (1) Ability to ferment rapidly and efficiently high sugar concentrations, combined with (2) tolerance to high temperatures and high nonsugar solids concentrations. Tolerance to high temperatures is especially important in locations where cooling water is at a premium. Distillery yeasts are usually strains of *Saccharomyces cerevisiae* and are commonly maintained in the laboratory on malt or molasses agar slants and transferred at monthly intervals.

Before use in the plant, cultures are usually successively propagated in the laboratory in two stages: (1) in small flasks containing 100 to 150 ml of 12 to 15% sterilized malt extract

medium, and (2) in 6-l flasks containing 4 l of sterilized mash, the sugar being supplied by malt extract and molasses. This second flask is provided with a side arm and suitable rubber-hose and pipe connections for aseptically transferring the yeast to the first plant stage.

The first plant stage, known as the preseed stage, may have a volume up to 300 gal. The mash for this stage and the following seed stage consists entirely of sterilized dilute molasses and inorganic nutrients, if necessary. The sugar concentration in these stages is usually 8 to 12%. The final seed stage contains up to 10,000 gal of mash and is used to inoculate the final fermentor. The final fermentors have volumes up to 125,000 gal, although some larger vessels are also in use in certain plants. Usually, an inoculum of 2 to 4% by volume of active seed yeast is used for inoculation of the final fermentors. While no reduction in efficiency is noted if a rather completely worked-out seed is used, provided it has been cooled, it is usually considered good practice to use the seed yeast before more than two-thirds of the sugar has been fermented.

Considerable plant equipment and operating time is saved by the process of "seeding-back," which consists of adding sterilized mash to a relatively large volume of partly fermented active pure-yeast culture. For example, from a 10,000-gal seed vessel 5,000 gal of yeast culture can be drawn off every 8 hours and the volume brought back to the original 10,000 gal with fresh, sterilized and cooled mash. As a rule, this process of seeding-back cannot be continued longer than 2 or 3 days without the yeast weakening and suffering a corresponding reduction in efficiency in the fermentor stage. At the end of this time, the seeder should be started fresh from a laboratory culture.

Legg[23] claims an increased yield from molasses, especially off-grade molasses, by the use of a mixture of grain and molasses yeast types in the fermentation.

## THE PLANT FERMENTATION PROPER

### Mash Preparation

For the plant mash, molasses is diluted with water to give a sugar concentration of 14 to 18% and pumped directly into the fermentor. This mash is usually not sterilized, although in certain

cases it has been pasteurized with a resultant slight increase in efficiency. The fermentor is seeded when it is one-eighth to one-fourth full with a large volume of active yeast (2 to 4% of the final volume) to allow development of the yeast during the entire filling period, which may amount to 8 hours, and to avoid growth of contaminating organisms during this period. Figure 19 illustrates the method of filling fermentors in a molasses distillery.

FIGURE. 19. *Operating Floor of Fermenting Room* (Courtesy—U. S. Industrial Chemicals, Inc., New York, N. Y.)

### Acidity

The mash is adjusted to a pH of 4 to 5 with sulfuric acid, usually 1 to 2 gal of acid being required per 1,000 gal of mash. Although the optimum pH for maximum efficiency varies with the molasses used,[16,33] an initial pH of 4.8 to 5.0 is usually considered best. Hydrochloric or lactic acid may be used in place of sulfuric acid.

### Nutrients

Blackstrap molasses usually contains enough yeast nutrients to give a fast, efficient fermentation. In some cases, however, it is desirable to add small quantities of ammonium salts, such as am-

monium sulfate, to the mash to increase the rate and efficiency of the fermentation. In such cases, the amount of ammonium sulfate added varies between 0.5 and 3 lb per 1,000 gal of mash, depending on the molasses used, the optimum amount being determined by laboratory test fermentations. Additional phosphate is rarely needed in a blackstrap molasses fermentation.

High-test molasses is more difficult to ferment than blackstrap, since it contains a smaller quantity of yeast nutrients and is relatively poorly buffered. From 3 to 6 lb of ammonium sulfate per 1,000 gal is required and the addition of some phosphate is beneficial.[32] Aqua ammonia is commonly added at intervals during the fermentation of high-test molasses to maintain a desirable pH and stimulate the fermentation. The fermentation of high-test molasses is greatly facilitated if the procedure known as "slopping-back" is employed. Slopping-back consists of adding to the molasses mash cooled, dealcoholized stillage from a previous fermentation. With blackstrap molasses, an increased yield is usually obtained when 10 to 20% of the mash volume consists of stillage from a previous fermentation. When a volume greater than 30% is used, the alcohol yield is generally adversely affected. With high-test molasses, however, up to 50% of the mash volume may consist of stillage. Under these conditions, the mash is well buffered, yeast nutrients liberated from the previous crop of yeast during distillation become available, and a rapid, efficient fermentation is obtained. Slopping-back is employed extensively in plants where slop must be evaporated before disposal since it results in an appreciable lowering of evaporation costs. Autolyzed yeast has been recommended by Owen,[27] Shukla,[47] and Blaisten[8] as a nutrient.

## Fermentation Temperatures

Fermentors are usually set at a temperature between 70° and 80°F, and are held at 90° to 92°F by the use of water sprays on the tank, internal cooling coils, or by circulation of the mash through external coolers. It is desirable to maintain the temperature of the mash below 95°F. The amount of heat liberated during the fermentation agrees with the theoretical value:[35]

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 + 26.0 \text{ Calories.}$$

The heat produced from a fermentation involving 150,000 lb of

sugar is 39,000,000Btu. If the fermentors are not cooled, the temperature of the mash will rise as much as 30°F.

### Fermentation Time

Fermentation begins promptly after the fermentor is filled and is usually active after 2 to 4 hours. Fermentation times vary with the molasses used, but a Cuban blackstrap fermentation is usually complete in 36 hours while molasses from Puerto Rico and Java require 48 and 72 hours, respectively. After fermentation is complete, the fermented mash known as "beer" and containing 6 to 9% alcohol is pumped to a temporary storage tank or "beer-well" prior to distillation.

## CONTAMINATION PROBLEMS

The mash in alcoholic molasses fermentation is usually not sterilized, the chief defense against contaminants being the adjustment of the acidity to pH 5 or slightly below. Many contaminants will not grow readily at such pH levels. The fermentation is usually so vigorous that anaerobic conditions are quickly established and the alcohol produced tends to inhibit those lactic and butyric organisms that do develop. Molasses itself usually contains a relatively small flora consisting of spores of molds, bacteria, and yeasts. Many common species of bacteria will not multiply to any extent in molasses mashes containing 15% sugars.

When back-slopping is employed, care must be taken to avoid contamination of the mash with bacteria from stillage lines and coolers, incrustations on valves, dead ends, etc., since these parts of the equipment can build up residues and thus act as foci of infection, harboring bacteria acclimatized to and capable of growing in molasses mashes. Good practice consists of daily washing and steaming of all mash and stillage lines and coolers. When not in use these lines are preferably held under steam pressure.

In some plants, ammonium bifluoride has been effectively used as a selective antiseptic. However, the use of such antiseptics is undesirable in those plants in which the stillages are to be used as livestock-feed ingredients.

Owen[28] has studied the effects of bacterial contamination on the alcohol efficiency in molasses fermentation and concluded that

contamination has a small but demonstrable effect on alcohol yields.

The yeast fermentation of molasses is a biologically stable process and is not subject to bacteriophage or other phage attacks. As a matter of interest, it has been suggested to add bacteriophages to the alcohol fermentation to control contaminants.[14]

## ANALYTICAL METHODS

Extensive analytical work is involved in the operation of a molasses alcohol plant. A typical control laboratory in an industrial alcohol plant is shown in Figure 20.

FIGURE 20. *Control Laboratory in an Industrial-Alcohol Plant* (Courtesy—U. S. Industrial Chemicals, Inc., New York, N. Y.)

Alcohol yields are obtained by distillation of a given volume of finished beer and collection of a definite volume of condensate

on which alcohol determinations are made by use of specific-gravity or refractive-index measurements. Sugar input is determined by standard methods using Fehling's solution. The Lane-Eynon volumetric procedure is rapid and convenient and is suitable for use in determining the sugar in the original mash. Routine laboratory tests are made to determine the optimum pH, proper amount of slop-back, and optimum nutrient additions for each batch of molasses segregated for plant use. These small-scale control fermentations are a reliable guide to good plant operation. Little or no progress has been made in controlling the fermentation of molasses solely on the basis of its chemical characteristics.

## FERMENTATION YIELDS

The most satisfactory basis for expressing yields is the fermentation efficiency. This figure is the ratio of the actual alcohol produced to the amount to be expected theoretically on the basis of the sugar contained in the molasses. The theoretical production is usually considered as 95% of the alcohol to be expected on the basis of the chemical transformation of sugar to alcohol. This allows 5% of the sugar for the production of yeast cells, and the by-products of fermentation, such as glycerol, succinic acid, etc.

The work originally done by Pasteur on the products of alcoholic fermentation serves very well as a practical guide for setting the maximum yield obtainable. He found that the fermentation of invert sugar yielded the following products expressed as percentage of sugar fermented:

| | % |
|---|---|
| Ethyl alcohol | 48.4 |
| Carbon dioxide | 46.6 |
| Glycerol | 3.3 |
| Succinic acid | 0.6 |
| Cellulose | 1.2 |

Stoichiometrically, one molecule of a six-carbon sugar would give two molecules of alcohol and two molecules of carbon dioxide, corresponding to 51.1% and 48.9%, respectively. The 48.4% alcohol obtained by Pasteur in actual experiments is very close to 95% of the amount to be expected on the basis of the chemical transformation of sugar to alcohol. It may be noted that Pasteur's alcohol yield is obtainable if pure sucrose is added to fully fermented

and dealcoholized molasses stillage and this solution is then seeded with yeast. In calculating the yield, the sucrose must, of course, be expressed as the corresponding invert sugar.

Alcohol efficiencies from blackstrap molasses, using conventional fermentation procedures, average 86 to 90% when calculated on the total invert sugar charged to the fermentor. This figure varies with the amount of nonfermentable sugar present in the molasses. An efficiency of 95% is obtained from the sugar fermented. When high-test molasses is used, efficiencies up to 93 or 94% on the total invert sugar charged to the fermentor are frequently obtained.

The quantity of blackstrap molasses required to produce 1 gal of 190° proof alcohol varies between 2.3 and 2.7 gal, depending on the sugar content and efficiency of the fermentation.

## YIELDS AND RECOVERY OF BY-PRODUCTS

### Fusel Oil

The high-boiling fraction (90° to 150°C) obtained in the distillation of fermentation beers is known as fusel oil. Fusel oil from molasses is a mixture of isopropyl and *n*-propyl, isobutyl and *n*-butyl, isoamyl and *d*-amyl alcohols. Over 50% of the total fusel oil consists of isoamyl and *d*-amyl alcohols. Analysis of fusel oil from molasses is given by Jacobs,[19,20] Kumamoto,[22] and Swenarton.[49]

It is well known that ammonium salts depress the yield of fusel oil, since yeast preferentially uses the ammonium ion directly rather than deaminating amino acids. The use of acid to adjust the pH of the mash likewise decreases the fusel oil yield. However, the increase in fusel oil yield in molasses fermentations not adjusted to the optimum pH usually does not compensate for the reduced ethyl alcohol efficiency due to fermenting at an unsuitable pH level.

### Carbon Dioxide

Carbon dioxide is available in large quantities as a by-product of the fermentation industry. From every 100 lb of molasses sugar fermented, approximately 46 lb of alcohol and 44 lb of carbon dioxide are produced. Thus, from a fermentor of 125,000 gal capacity, charged with 25,000 gal of molasses, about 75,000 lb of carbon dioxide is formed. Up to 80% of this can be recovered, the amount depending on the market conditions and seasonal

demand for carbon dioxide in the solid form or in cylinders for carbonating beverages.

Carbon dioxide is collected from closed fermentors after a vigorous fermentation has set in and the dissolved gases and air have been purged from the fermentor. The gas at this stage while relatively pure (99.5%) contains traces of entrained molasses solids, aldehydes, alcohol, and minute amounts of other impurities. These are responsible for an odor which must be removed before the gas can be utilized to carbonate beverages or be processed into solid carbon dioxide. Two general purification methods are used: the Backus process[5,6] and the Reich[37] process. The Backus process depends primarily on a direct adsorption of the impurities, while in the Reich process, the impurities are first oxidized and then removed by adsorption or absorption. Carbon dioxide is commonly purified as follows: (1) The gas is washed in a water scrubber which removes entrained solids and all but traces of aldehydes and alcohols. Some 0.5 to 1% of the total alcohol produced in the fermentation is recovered by distillation of the carbon dioxide wash water. (2) Water is removed from the gas by an acid scrubber containing 66° Baumé sulfuric acid and this is followed by (3) passage through an activated-carbon tower for removal of odors. The most troublesome impurities in solid carbon dioxide are odors originating from the molasses and lubricating oils used in the compressors. Gaseous carbon dioxide is compressed to form liquid carbon dioxide in two- or three-stage compressors, depending on the refrigerant used. By suitably rapid evaporation of liquid carbon dioxide, the solid is formed as a snow which may be compressed into cakes for use as "dry ice."

### Uses of Carbon Dioxide

Solid carbon dioxide is used primarily as a refrigerant for cooling and shrinking machine parts and for reliquefaction after transport to make liquid carbon dioxide. Liquid carbon dioxide is used extensively for carbonating beverages, in fire extinguishers, in food preservatives, in refractories, and in the chemical industry. A detailed treatment of carbon dioxide recovery method, uses, and sources is given in the monograph by Quinn and Jones.[34]

### Recovery of Carbon Dioxide

Of the total carbon dioxide produced in a fermentor, only 70 to 80% can be recovered as liquid or solid carbon dioxide.[26,46]

## RESIDUES AND WASTES

After distillation and recovery of alcohol from the beer, the still residue, known as stillage, contains 7 to 10% solids. The profitable use and disposal of this residue is still not completely worked out and is a problem of the industry.[11,48]

When conditions permit, the still residue is run into streams. However, it has a very high b.o.d. (biochemical oxygen demand) (18,000 to 22,000 ppm),[13,40] so that in localities where stream pollution is a factor, dumping it in this manner is prohibited by law. In such cases, the residue must be disposed of by other methods. The analytical breakdown of a typical stillage on a dry basis is given in Table 13.

TABLE 13. ANALYSIS OF DRIED-MOLASSES RESIDUE

| Ingredients | % |
|---|---|
| Mineral matter | 28.5 – 29.0 |
| Sugars (copper reducing substances) | 10.0 – 12.0 |
| Proteins | 8.0 – 10.0 |
| Volatile acids | 1.0 – 2.0 |
| Gums | 20.0 – 22.0 |
| Combined lactic acid | 4.0 – 5.0 |
| Other combined organic acids | 1.0 – 2.0 |
| Glycerol | 5.0 – 6.0 |
| Wax, phenolic bodies, lignin, glucosides, etc. | 22.0 – 12.0 |

The two major uses to which the still residues have been put are: (1) livestock feed, and (2) source of fertilizer ingredients.

## USE OF MOLASSES RESIDUE AS INGREDIENT IN LIVESTOCK FEEDS

For use in stock feeds, the dilute molasses residue is evaporated in vacuum evaporators to 45 to 50% solids. At this concentration, the evaporated residue will keep and is not too viscous to handle. It has been used in large quantities in the past few years as a molasses substitute in stock feeds, primarily for rations for dairy cattle. A typical analysis of evaporated molasses residue is presented in Table 14.

Evaporated molasses fermentation residue, containing all the growth factors present in yeast and molasses, has been used in large quantities in dairy rations. The evaporated molasses residue is also effective as an antidusting agent in feed mixing and handling,

TABLE 14. ANALYTICAL FIGURES ON EVAPORATED-MOLASSES RESIDUE

| *ANALYSIS, WET BASIS* | | % |
|---|---|---|
| Moisture | | 54.67 |
| Solids | | 45.33 |
| Protein | | 6.95 |
| Ash | | 10.93 |
| Gums | | 10.40 |
| Sugars (copper-reducing substances) | | 5.30 |
| Glycerol | | 2.60 |
| Lactic acid | | 2.70 |
| Fat | | 0.00 |
| Fiber | | 0.30 |
| Wax, lignin, glucosides, phenolic bodies, organic acids, etc. | | 6.15 |
| *ASH ANALYSIS, WET BASIS* | | % |
| Silica | | 0.4 |
| Iron | | 0.08 |
| Aluminum | | 0.09 |
| Calcium | | 1.4 |
| Magnesium | | 0.7 |
| Sulfur | | 1.4 |
| Phosphorus | | 0.3 |
| Sodium | | 0.5 |
| Potassium | | 3.4 |
| Chlorine | | 1.3 |
| Manganese | | 0.002 |
| Iodine | | 0.0014 |
| Copper | | 0.017 |
| *VITAMIN ANALYSIS, WET BASIS* | *μg per g* | *mg per lb* |
| Nicotinic acid | 21 | 9.53 |
| Pyridoxine | 30 | 13.62 |
| Pantothenic acid | 39 | 17.71 |
| Biotin | 1.5 | 0.68 |
| Folic acid | 0.3 | 0.135 |
| Riboflavin | 8 | 3.63 |

being about equal to molasses in this respect. It can be used to supplement molasses in mixed feeds or even as a substitute. As with molasses, consideration must be given to the high mineral content and the levels used kept below the point at which laxative effects became evident. A limited amount of evaporated molasses residue has been spray dried and used at low levels in poultry rations.

Small quantities of evaporated molasses residues have found use in foundry core binders and in briquet binders.[24]

### Use of Molasses Residues as Fertilizer

Molasses residues contain considerable quantities of potash salts, nitrogen compounds, and small quantities of phosphates. The dilute residue can be applied directly to the soil with beneficial effects. However, the low unit value and high transportation cost preclude any large-scale direct use of this residue as a liquid fertilizer. Diaz d'Arce[13] has described a process which involves biologically reducing the b.o.d. and using the dilute slop in irrigation water. For many years (1919-1940), large quantities of evaporated molasses residues were burned for their potash values at one large eastern distillery.[12] This subject is also discussed by Owen.[25,26] With the development of a domestic potash industry and falling potash prices, the process has become obsolete since the values obtained no longer pay the evaporation costs.

Reich[38,40,41] has obtained patents on a process in which the evaporated slop is neutralized with potassium carbonate, dried, and carbonized at high temperatures. After the char is washed, an activated carbon having high decolorizing properties is said to be obtained. Potash and tar are by-products of this process. No production figures or costs have appeared in the literature on this process.

## MINOR VARIATIONS OF THE FERMENTATION PROCESS

### Continuous Processes

Since a continuous fermentation process would allow the use of smaller fermentors and supplementary equipment in a distillery, it has long been of interest to the industry.

Continuous fermentation methods have been used successfully on waste sulfite liquor in Europe. In "fixed yeast" methods, the yeast cells are supported on chips, etc., the liquor flowing up through the bed and out the top of the fermentor. In the "bottom yeast" method, yeast left at the bottom of the fermentors is used to seed incoming mash. Several fermentors are often connected in series.

Since sulfite liquors are sterile and even antiseptic, continuous fermentation is possible and desirable with such a substrate. With more readily fermentable substances, such as molasses, any contamination is cumulative and soon spreads throughout the system,

reducing yields. A continuous fermentation scheme, involving a series of tanks in which the mash overflows from one tank to another, has been tried on a plant scale and in the past has given poor results. Interest in this scheme has recently been revived by Alzola.[1,2] Owen[29] described a laboratory apparatus involving down flow of the mash through a series of decks. A second method of continuous fermentation involves the use of one heavily seeded fermentor provided with continuous feed and draw-off systems. Short fermentation times are involved. Such a system was described by Bilford, Scalf, Stark, and Kolachov.[7]. These authors gave the results of laboratory-scale continuous fermentations of molasses with runs of up to a 31-hour duration. They found that mechanical or carbon dioxide agitation of the fermenting mash was essential and that the optimum through-put rate was between 19 and 25% of the mash volume per hour. A similar process has been described by Blaisten[8] in which molasses mash, containing 12% sugar, was maintained at a pH of 4 to 5 with ammonium hydroxide and the yeast count kept above 500 million per milliliter. Autolyzed yeast was added as a nutrient and slow mechanical stirring was employed. A high efficiency was claimed with a 15% hourly draw-off.

Recently Adams and Hungate,[1a] described a method for predicting cycle times for continuous fermentations. The cycle time is the time needed for replacing completely the original fermentation medium. The length of the continuous fermentation cycle, at any specific yeast population, can be calculated from batch-growth curves. By drawing tangents to the growth curve at desired levels, it was possible to calculate the theoretical cycles by dividing the total yeast population, at the point to which the tangent was drawn, by the slope of the tangent. The method was applicable to all media tested except those that had a nutritional deficiency.

Karsch[21] has patented a continuous process in which the yeast is separated from the beer and returned to the liquor to be fermented. No data on large-scale continuous fermentation methods for molasses are available in the literature.

## The Usines de Melle Process

In the fermentation of blackstrap molasses, the alcohol efficiency on the fermented sugar averages only about 95% of theory and this is attributed, in part, to the fact that 2 to 3% of the sugar

fermented is utilized in the production of yeast-cell protoplasm and is, therefore, not available for alcohol fermentation. The loss is said to be avoided by the Melle process[9] which consists essentially in centrifuging out the yeast after the fermentation is complete and reusing the same yeast in the next fermentation cycle. In the normal alcoholic fermentation, the yeast population is 50 to 60 million cells per milliliter, although higher counts are frequently found, especially if large seedings are used. By reusing the yeast, it is claimed for the Melle process that the "cellular saturation point of the solution" is established at once and all the fermentable sugar is then available for alcohol production. In commercial practice, the problem of contamination in the Melle process is taken care of in two ways: (1) The bacterial contaminations in the beer, being lighter than yeast, are to some extent concentrated in the centrifuge effluent and fewer bacteria remain in the reused yeast slurry. (2) The centrifuged yeast is freed of bacteria by washing with four to five times its volume of water, and holding at pH 2 for 4 hours in the presence of carbon dioxide.[10]

Lagomasino[22a] described the Melle-Boinot alcoholic fermentation method in some detail. Formation of new yeast cells at the expense of sugar was avoided almost completely and alcohol yields as high as 97% of theoretical were obtained. The yeast was separated from the fermented beers before distillation and immediately reused. Advantages claimed for the process are: Beers containing 9 to 10% alcohol are obtained. The use of sugar for yeast production is largely eliminated. Fermentation time is reduced to 12 to 18 hours. Rapid alcohol formation inhibits the growth of foreign organisms. A higher degree of cleanliness is maintained in the still and "vat lees" are noticeably reduced. About 60% of the stillage can be used for diluting the molasses for fermentation while the remainder, low in organic matter, offers less of a disposal problem.

The Melle process has been successfully used in over one hundred foreign distilleries and in sulfite-liquor plants here and abroad. It has not been used in the United States in the molasses-fermentation industry because economic conditions have not been such as to justify the cost of operation of the centrifuges necessary to handle the large volume of mash involved. Also, the successful use of centrifuges requires some preliminary clarification of the molasses which has not been economically practicable.

### The Two-Stage Process

Another solution to the problem of obtaining the yeast necessary for the fermentation at a small charge is found in the two-stage process of Hildebrandt and Erb.[17,18] This process is based on the observation that molasses distillery stillage contains sufficient sugar, derived by hydrolysis of unfermentables during distillation, to develop a good yeast crop when grown aerobically. Under ideal conditions of aeration and agitation, 200 to 400 million yeast cells per milliliter can be developed in distillery stillage, but under actual plant conditions in normal molasses fermentations, about 50 million cells per milliliter are obtained. After a yeast crop is grown on stillage, molasses is mixed with the pregrown yeast and the alcoholic fermentation proper is allowed to proceed in the second stage under anaerobic conditions. In this way, the advantages of a cost-free pregrown yeast crop are obtained without any capital investment in centrifuges. The two-stage method of fermentation is especially advantageous in those plants where the disposal of residues is a problem, since the rate of slopping-back can be increased and evaporation costs reduced by using this process. The process is also very useful in fermenting high-test molasses since very high slopping-back rates can be used.

### Processes for Fermenting High-Concentration Mashes

Rao, Bhimeswar, and Sreenivasaya[36] reported a new yeast which gave alcohol concentrations of 12 to 14% in fermented-molasses beers. In two distilleries in India, they claimed the process using this yeast resulted in 50% reduction in cost.

Arroyo[4] described a fermentation technique for producing alcohol from high-concentration molasses mashes. He claimed that by this process 50 to 100% more molasses could be processed, resulting in a corresponding increase in production. The procedure involves addition of proper quantities of ammonium sulfate and calcium superphosphate to a thick mash having a Brix density of 55 to 60° and heating this mash for 8 hours at 80°C and a pH of 4.5 to 5.2. During this period, considerable inversion of sucrose occurs and the impurities of the thick mash settle at the base of the settling tank. The sediment is removed, the mash diluted to a Brix density of 20 to 23° and fermented in closed fermentors, employing 10 to 15% by volume of yeast inoculum. The total

mash is introduced into the fermentor in three increments, fermentation time being 30 to 36 hours. Depending on the quality of the blackstrap molasses employed, the fermented beers had alcohol concentrations in the range of 10 to 13.5% by volume. No data were given as to the efficiency of carbohydrate conversion.

Owen[30] studied the Arroyo process and found that the clarified mash fermented more slowly, but gave higher alcohol efficiency than either the sludge or untreated molasses. He attributed the slower fermentation of the clarified mash to removal of colloids which tend to accelerate fermentation. The Reich[39,42] process of clarification was not investigated by Owen, but may be expected to give similar results. The Reich process consists of heating molasses solutions of 25 to 60° Brix to about 70°C after addition of sulfuric acid to a pH of 3 to 6.

## COMPETITIVE PROCESSES FOR ALCOHOL MANUFACTURE

Industrial alcohol can also be made by fermenting starchy materials, such as potatoes, grains, cassava, etc., wood hydrolyzates and sulfite waste liquors. These processes are discussed in Chapters 2, 4 and 5 of this book. Alcohol is also produced synthetically from ethylene, derived principally from petroleum-refinery waste gases, by the way of ethyl hydrogen sulfate, and from carbon monoxide and hydrogen, derived from natural gas or coal by means of hydrocarbon synthesis methods.

Production of synthetic alcohol from ethylene has more than tripled in the last decade due to increased demand during the World War II emergency and the prevailing high price of molasses sugar, so that the present synthetic-alcohol industry can supply well over half of the normal demand. Since the cost of alcohol from ethylene is probably under 20 cents per gallon,[50] it would be necessary for the price of molasses to fall to 3.5 to 4 cents per gallon to render the fermentation industry truly competitive. It remains to be seen how the synthetic and fermentation industries will adjust themselves to this economic situation. Since the potential raw materials for the fermentation process are, under normal conditions, by-products, it is likely that a portion of the alcohol used industrially will continue to be made by fermentation in spite of the present unfavorable economic situation. An interesting survey of current

trends in industrial alcohol production, uses, prices, processes, and raw materials has recently appeared.[3a]

## BIBLIOGRAPHY


1a. Adams, S. L., and R. E. Hungate, *Ind. Eng. Chem.*, **42**, 1815 (1950).
1. Alzola, F., *Mem. 14th conf. anual, Asoc. tec. azucar. Cuba*, **1940**, 323.
2. Alzola, F., U. S. Patent 2,371,208 (1945).
3. Anon., *Ind. Eng. Chem.*, **40**, 990 (1948).
3a. Anon., *Chem. Eng. News*, **29**, 4931 (1951).
4. Arroyo, R., *Sugar J.*, **11**, No. 8, 5 (1949).
5. Backhaus, A. A., U. S. Patent 1,493,183 (1924).
6. Backhaus, A. A., U. S. Patent 1,510,373 (1924).
7. Bilford, H. R., R. E. Scalf, W. H. Stark, and P. J. Kolachov, *Ind. Eng. Chem.*, **34**, 1406 (1942).
8. Blaisten, R. J., *Arch. farm bioquím. univ. nacl. Tucamán*, **1**, 353 (1945).
9. Boinot, F., U. S. Patent 2,063,223 (1936).
10. Boinot, F., U. S. Patent 2,230,318 (1941).
11. Boruff, C. S., *Ind. Eng. Chem.*, **29**, 602 (1947).
12. Broughton, L. B., H. L. Marshall, and N. C. Thornton, *Md. Agr. Expt. Sta. Bull., No. 300* (1928).
13. d'Arce, B. D., *Mem. Asoc. técnicos azucar. Cuba*, **20**, 517 (1946).
14. Drel-Gitis, E., *Spirto-Vodochnaya Prom.*, **15**, No. 10, 14 (1938).
15. Erb, C., and F. W. Zerban, *Sugar J.*, **11**, No. 12, 5 (1949).
16. Hildebrandt, F. M., *Ind. Eng. Chem.*, **21**, 779 (1929).
17. Hildebrandt, F. M., and N. M. Erb, U. S. Patent 2,169,244 (1939).
18. Hildebrandt, F. M., and N. M. Erb, U. S. Patent 2,169,245 (1939).
19. Jacobs, P. B., *U.S.D.A., Bur. Agr. Ind. Chem.*, AIC 95, 77 (1945).
20. Jacobs, P. B., "Industrial Alcohol," Washington, D. C., *U.S.D.A. Misc. Publ. No. 695*, 1950.
21. Karsh, W., U. S. Patent 2,155,134 (1939).
22. Kumamoto, S., *J. Chem. Soc. Japan*, **53**, 30 (1932).
22a. Lagomasino, J. M., *Intern. Sugar J.*, **51**, 338 (1949).
23. Legg, D. A., U. S. Patent 2,419,960 (1947).

24. Moore, W. C., and H. A. Myers, *Ind. Eng. Chem.*, **19**, 147 (1927).
25. Owen, W. L., *Facts about Sugar,* **33**, No. 6, 45 (1938).
26. Owen, W. L., *Blackstrap Molasses as Raw Material for Biochemical Industries,* New York, Russell Palmer, 1939.
27. Owen, W. L., U. S. Patent 2,272,982 (1942).
28. Owen, W. L., *Sugar,* **41**, No. 6, 46 (1946).
29. Owen, W. L., *Sugar,* **43**, No. 2, 36 (1948).
30. Owen, W. L., *Sugar,* **43**, No. 4, 37 (1948).
31. Owen, W. L., *Intern. Sugar J.*, **51**, 81 (1949).
32. Owen, W. L., and T. S. Chen, *J. Chem. Eng. China,* **4**, 212 (1937).
33. Perdoma, E. V., *Mem. Asoc. técnicos azucar. Cuba,* **20**, 467 (1946).
34. Quinn, E. L., and C. L. Jones, *Carbon Dioxide,* Monograph Series, Am. Chem. Soc., New York, Reinhold, 1936.
35. Rahn, O., *Physiology of Bacteria,* Philadelphia, Blakiston, 1932.
36. Rao, S. R. A., B. Bhimeswar and M. Sreenivasaya, *Rept. Proc. 4th Intern. Congr. Microbiol.*, **1947**, 562 (1949).
37. Reich, G. T., U. S. Patent 1,519,932 (1924).
38. Reich, G. T., U. S. Patent 2,148,579 (1939).
39. Reich, G. T., *Trans. Am. Inst. Chem. Engrs.*, **38**, 1049 (1942).
40. Reich, G. T., *Trans. Am. Inst. Chem. Engrs.*, **41**, 233 (1945).
41. Reich, G. T., *Ind. Eng. Chem.*, **37**, 534 (1945).
42. Reich, G. T., U. S. Patent 2,464,611 (1949).
43. Sattler, L., and F. W. Zerban, *Ind. Eng. Chem.*, **34**, 1180 (1942).
44. Sattler, L., and F. W. Zerban, *Ind. Eng. Chem.*, **37**, 1133 (1945).
45. Sattler, L., and F. W. Zerban, *Ind. Eng. Chem.*, **41**, 1401 (1949).
46. Sherwood, I. R., *Austral. Chem. Inst. J. & Proc.*, **14**, 221 (1947).
47. Shukla, J. P., *Proc. Sugar Tech. Assoc. India,* **12**, II, 99 (1943).
48. Streetar, H. W., *Proc. Ind. Waste Utiliz. Conf., Purdue Uni.*, **1**, 150 (1944).
49. Swenarton, J., *Science,* **70**, 554 (1929).
50. Tousley, R. D., *Chem. & Met. Eng.*, **52**, No. 10, 120 (1945).
51. Zerban, F. W., "The Color Problem in Sucrose Manufacture," New York, Sugar Research Foundation, Inc., *Tech. Rept. Series No. 2*, 1947.

CHAPTER 4

# ALCOHOLIC FERMENTATION OF SULFITE WASTE LIQUOR

*Joseph L. McCarthy*

Sulfite waste liquor, sometimes called "waste sulfite liquor," "sulfite liquor," or "sulfite spent liquor," is the aqueous effluent from the "sulfite" process for manufacturing cellulose or pulp from wood. This liquor contains sugars and industrial production of ethyl alcohol by fermentation of some of these sugars has been conducted for many years on a considerable scale in Northern Europe. Three major plants are now operating in North America.

The sulfite pulping process, introduced about 1867 apparently independently by B. C. Tilghman, C. D. Ekman, and A. Mitscherlich,[78,137] is conducted by treating chips of wood at elevated temperature with an aqueous solution of sulfurous acid containing a cation, such as calcium, magnesium, sodium, or ammonium. During this reaction, most of the lignins and hemicelluloses are brought into solution and fibrous wood cellulose or sulfite pulp is left behind which can be separated and bleached for paper, rayon, tire cord, explosives, and other uses. The remaining sulfite waste liquor contains organic substances amounting to more than half of the weight of the original wood and most of the inorganic chemicals used in the pulping reaction. A practical utilization of this liquor has long been sought for economic and conservation reasons and

because, if discharged into water courses, it may cause depletion in dissolved oxygen and thus interference with aquatic life.

The utilization of sugars in sulfite waste liquor by fermentation to produce alcohol was apparently first proposed and patented about 1878 by A. Mitscherlich.[67] However, it was not until about 1907 in Sweden that the first experimental plants were built, one under the supervision of P. G. Ekström at Skutskär and the other under J. H. Wallin at Koppmanholmen.[76,80] The Wallin plant was closed the following year, but in 1909 the alcohol plant at Skutskär was expanded to process the entire sulfite waste liquor produced by the mill.

Clementson[40] has described the steps of the Ekström process which may be summarized as follows: (1) collecting hot sulfite waste liquor from the digesters; (2) neutralizing the liquor with limestone and stirring with steam; (3) settling sediment from the liquor at 158° to 175°F; (4) cooling the liquor in a tower to about 86°F; (5) adding yeast and nutrients, such as ammonium sulfate, superphosphate or phosphoric acid; (6) fermenting the liquor in tanks; (7) degassing carbon dioxide from the fermented liquor which contains about 1% alcohol by volume; and (8) distilling to recover alcohol at about 95% concentration. For a mill manufacturing 20,000 tons of sulfite pulp a year, with recovery of 1,455 gal of sulfite waste liquor per ton of pulp produced, Clementson in 1921 estimated a yield of about 11 gal of 95% ethyl alcohol per 1,000 gal of waste liquor handled, or about 16 gal of alcohol per ton of pulp manufactured, and a total cost of 21.4¢ per gallon of ethyl alcohol produced.

Based on the success of the Skutskär operation, the Ethyl Company, with the patents of Ekström, promoted the establishment of new plants at Kvarnsveden and Bergvik in 1911, then at Gotha in 1916. Six additional plants were put up in 1918, another six in 1919, and five more in 1920, to make a total of twenty-one plants established in Sweden by 1921 and capable of producing annually some 5,300,000 gal of 95% ethyl alcohol. A few plants were set up in other countries. However, further growth of the industry has been fairly slow for economic and political reasons which have been well summarized by Hansen.[97]

The Ekström process was also used in the United States in a full-scale alcohol plant built in 1914 by the West Virginia Pulp and Paper Company at Mechanicsville, New York.[73,80,185] However

this plant did not prove to be profitable, with a market price of 50¢ per gallon of alcohol,[187] and the plant was operated only intermittently over about 25 years, sometimes with molasses added to the sulfite liquor. A modified process was evolved by C. Marchand[139] and studied in 1915 on a small pilot scale at the Oregon City, Oregon, plant of the Crown Willamette Paper Company;[199] the process was further tested in 1916 in an experimental plant of the Kimberly-Clark Corporation at Kimberly, Wisconsin, but full-scale production was not undertaken.[97] Experimental study of the McKee process[73,138] was conducted about 1920 by the Hammermill Paper Company at Erie, Pennsylvania. Further major developments did not take place until during or after World War II.

## ORIGIN AND CHARACTERISTICS OF SULFITE WASTE LIQUOR SUGARS AND RELATED SUBSTANCES

### The Carbohydrate Components of Wood

Most woods consist principally of about 50% cellulose, some 25% lignins, and around 25% of a not very well defined group of carbohydrates, called hemicelluloses, which hydrolyze and dissolve during the sulfite pulping process to yield most or all the sugars in sulfite waste liquor which can be fermented to alcohol. Investigations of the nature and yield of the sugars obtained by total and partial hydrolysis of wood have shown that hemicelluloses are built up of the hexose sugars: glucose, mannose, galactose, and perhaps fructose; and the pentose sugars: xylose, and arabinose.[216] (See also Tables 21 through 24 of Chapter 5.) Of these sugars, glucose, fructose, and perhaps galactose are fermentable by yeasts. The pentoses are not fermentable. Since softwoods, or gymnosperms, contain only about 10% of pentosans compared with about 20% in hardwoods or angiosperms, sulfite waste liquor resulting from softwoods is favored for fermentation to produce alcohol, while liquors from hardwoods are sometimes employed for production of *Torulopsis* fodder yeasts because these yeasts can grow on pentose sugars and some aliphatic carboxy acids as well as on hexoses. The "theoretical" maximum yield of alcohol which might be produced from a softwood sulfite waste liquor has been calculated from the composition of wood to be around 40 to 50 gal per ton of sulfite pulp produced (see Table 15).

TABLE 15. REPORTED YIELDS OF 95% ETHYL ALCOHOL FROM SULFITE WASTE LIQUORS

| | *Yield for Various Types of Pulp* | | | | |
|---|---|---|---|---|---|
| | Strong | Easy bleaching | Viscose | Fodder | Literature reference |
| Condition or plant | U. S. gallons per ton of air-dry pulp | | | | |
| Theoretical yield | 43.3 | 47.2 | —— | —— | 24 |
| Theoretical yield | —— | 48.1 | —— | —— | 77 |
| Theoretical yield | 40.4 | 43.0 | —— | —— | 191 |
| Average, no washing | 8.8 | —— | 17.7 | —— | 77 |
| Average for Sweden | —— | 10.8 | —— | —— | 191 |
| Average for Germany | 9.6 | 12.0 | —— | —— | 115 |
| Average, good washing | 11.3 | —— | 20.2 | —— | 77 |
| Good operation | 16.9 | 19.6 | —— | —— | 111 |
| Best in Sweden, 1939 | —— | —— | —— | 26.3 | 111 |
| Two-stage pulping | —— | —— | 21.5 | —— | 11 |
| Two-stage claim | —— | 27.8 | —— | 32.8 | 77 |
| Bergson's system | 19.2 | 28.8 | —— | —— | 24 |
| Bergson's maximum result | —— | —— | 39.6 | 42.0 | 24 |
| Ontario Paper Co. plant | 16.6 | —— | —— | —— | 176 |
| Puget Sound P. T. Co. plant | —— | 22.0 | —— | —— | 62 |
| Commercial Alc. Ltd. plant[a] | —— | (24.4) | —— | —— | 6 |

[a] Yield from Commercial Alcohol Ltd. plant is estimated from reported design capacity of alcohol plant at 9,000 gal per day and daily production of 365 tons of sulfite pulp.

Source: Data tabulated by Hanson[97] except for the last three items.

## Some Aspects of the Chemistry of Sulfite Pulping

Sulfite pulping is usually conducted at 130° to 140°C for 6 to 12 hours with an aqueous solution of sulfurous acid containing calcium ions in such proportions as to provide around 5 to 7% "free sulfur dioxide" and 1% "combined sulfur dioxide." By using increasingly intense conditions for delignification and hemicellulose removal, several different types of sulfite pulp can be produced for particular purposes, e.g., for papers, rayons, or fodder. Correspondingly different sulfite waste liquors result. The reaction is carried out batch-wise in large, acid-resistant, brick-lined vessels or digesters charged with wood chips and solution in the ratio of about 1:4 or 1:5. The digesters are heated directly or indirectly by steam.[137]

Under these conditions, a complex series of reactions takes place between wood constituents and sulfite pulping liquors. The nature of these reactions has been largely clarified by the important contributions of the Swedish investigators, Erik Hägglund,[83] H. Erdtman,[61] and many others.[33,119,137,208,216] Although incompletely understood, a likely course of the reactions is as follows: (1) The

acidic reagent hydrolyzes chemical linkages between lignin molecules and/or carbohydrates, but does not bring about extensive hydrolysis of the more resistant cellulose which is thus preserved in a fibrous state to be recovered as wood pulp; (2) the reagent brings about sulfonation of lignin which becomes soluble in water and, after hydrolysis, is dissolved; (3) the reagent provides calcium or another basic ion for buffering or neutralizing the strong lignin sulfonic acids as they are formed and thus avoids undesirable cellulose degradation; (4) the acidic reagent hydrolyzes hemicelluloses mostly into monomeric sugars some of which are fermentable; (5) Hägglund[89] suggests that the reagent, probably because of the presence of bisulfite ions, converts some monomeric sugars from aldoses to aldose bisulfite addition compounds and to sulfonic and aldonic acids.

The kinetics of acidic hydrolysis of hemicelluloses have not yet been treated fundamentally because of the lack of information on the structure of these polymers or copolymers and because of the at least partly heterogeneous character of hydrolysis as it proceeds during sulfite pulping. However, Sundman[196] has recently contributed some interesting results obtained by determining the dissolved sugars present at intervals during the process of sulfite pulping of spruce, pine, aspen, and birch woods. The first sugar to appear was arabinose, which was found when the reaction temperature was less than 100°C. Xylose and galactose appeared simultaneously with arabinose or a little later. Mannose was first found much later in the reaction at which time the temperature had reached about 130°C and glucose appeared afterward. Although all woods studied yielded sulfite waste liquors which, at some stage of the pulping reaction contained arabinose, xylose, galactose, mannose, and glucose, there were substantial quantitative differences, especially between the proportions of mannose and galactose from hardwoods versus softwoods. These variations seem to result from the combined effects of differences in the amounts of each sugar polymer in wood, the accessibility of the various linkages, the velocity of hydrolysis of linkages in the polymers, and the velocity of decomposition or conversion of the monomeric sugars in the acidic reaction mixture.

The influence of sugar-decomposition reactions is apparent in a somewhat similar study conducted by Höpner[106] under sulfite pulping conditions suited to produce pulp for rayon. He observed

that during cooking the concentrations of the various sugars increased to a maximum and then decreased because of decomposition. Maximum concentrations of pentoses and of galactose (0.4%) were reached in 10 hours. The maximum concentrations of mannose (1.6%) and of fermentable sugars (2.3%) were obtained after 13 hours of reaction. Hägglund, Heiwinkel, and Bergek[87] have estimated that fermentable-sugar decomposition may reach 40 to 60% under certain conditions.

The sugar decomposition products appear to result from the acidic reaction and from the effect of the bisulfite ion. Thus furfural is present in sulfite waste liquor in small amounts, although hydroxymethylfurfural has not been observed.

The interaction between sugars and ingredients of sulfite pulping liquors is thought[87] to proceed in at least two ways, i.e., to form bisulfite addition compounds and then aldonic acids, or to form stable sugar sulfonic acids in small amounts. A sugar sulfonic acid preparation was obtained by Hägglund, Johnson, and Urban[89] by heating glucose with sodium sulfite solution at pH 6. Although this product was later found to be a mixture of two sugar sulfonic acids,[13,102] study of properties indicates that these substances are stable in boiling dilute mineral acids and in cold dilute alkalis, are not fermented, and have apparently no influence on the fermentation of glucose.[89] The sugar bisulfite addition compounds, which can markedly inhibit fermentation, are converted in part to nonfermentable aldonic acids.[87,89]

## Some Developments in the Technology of Pulping

While calcium is the basic ion now commonly used in the sulfite pulping process, at least as good wood pulp can be manufactured by the sulfite process, using magnesium, ammonium, or sodium ions instead of calcium ions. Because of this fact and because magnesium, ammonium, or sodium sulfite waste liquors can be processed to yield heat and chemical recovery in some cases when this would be difficult or impossible with calcium liquor, there is considerable interest in sulfite pulping with bases other than calcium.[99,162,209]

A magnesium-base sulfite-pulping and recovery system, developed by Tomlinson and Wilcoxson,[203] and Hatch and associates,[98] is now being operated to produce about 285 tons of sulfite pulp per

day at the Longview, Washington, plant of the Weyerhaeuser Timber Company.[5] Magnesium sulfite waste liquor is collected, evaporated in multieffect equipment, and then the organic solids in the concentrated liquor are burned in specially designed furnaces to produce process steam. From the furnace, magnesium oxide is also recovered which, in water slurry, serves as an absorbent to recover sulfur dioxide from the stack gases. The recovered chemicals are reused in the pulping process. The greater water solubility of magnesium than calcium salts makes feasible sulfite pulping with as little as 2.5 lb of magnesium pulping liquor per pound of moisture-free wood (compared with about 4.5 lb of calcium pulping liquor) to yield a sulfite waste liquor relatively high in total solids and fermentable sugars, which can be evaporated or else treated to recover alcohol with much less input of heat than is otherwise possible.[100,155]

Ammonium-base sulfite pulping is in operation at Toten, Norway, by the Norek Hydro-Elektrisk Kvaelstofaktieselskab;[41] at Shelton, Washington, by Rayonier, Inc.; at Bangor, Maine, by the Eastern Manufacturing Company; and at Lebanon, Oregon, by the Crown Zellerbach and Soundview Pulp Companies.[210] At the Shelton installation, the ammonium sulfite waste liquor is evaporated and burned. Sodium-base sulfite pulping[4] is conducted at Stutskär, Sweden, by the Stora Kopparberg A. B., with recovery of alcohol, heat, and process chemicals from the sulfite waste liquor;[121] other recovery systems for sodium-base sulfite waste liquor have been also discussed.[27,136] Continuous sulfite pulping is being studied.[164]

The conditions of sulfite pulping which would give maximum yield of fermentable sugars and thus of alcohol were much considered in Sweden during the early part of World War II because it was thought that the use of alcohol as a motor fuel would be the most promising solution of problems arising from petroleum shortage.[81] Johansson,[111] for example, found that the maximum alcohol yield is obtainable from a sulfite waste liquor from production of an easy-bleaching (i.e., extensively delignified) pulp of secondary quality obtained in minimum reaction time, with minimum calcium in the pulping liquor; increased reaction time brings about such serious sugar decomposition that the alcohol yield is decreased although the degraded pulp can be used for feeding ruminants.

The return of some liquor from a partly completed pulping

reaction to a later one was proposed by Bergson[23,24] in order to increase the concentration of total solids and thus sugars in sulfite waste liquor and more recent studies by Hägglund, Stockman, and Löfström[95] indicate that sulfite waste liquor solutions may be obtained containing 55 g of sugars per liter. Hägglund,[19] in 1938, suggested the use of a two-stage sulfite pulping procedure, using only a small calcium ion concentration in the first stage to minimize sugar decomposition; this process is not used on the industrial scale. The various possibilities of increasing alcohol yields were set before the Swedish government by the Engineers Academy[59] and increased capacity was developed although limited demand for Swedish pulp caused many plants to convert to production of fodder pulp.[141]

Wood pulp is also produced on a large scale by the "kraft" or "sulfate" process, using an aqueous solution of sodium hydroxide and sodium sulfide to accomplish delignification.[137,177] In the presence of alkalis, soluble carbohydrates undergo degradation and rearrangement to yield products unsuitable for alcoholic fermentation. While ordinary kraft pulps are used for many purposes, on xanthating, they yield solutions which are difficult to filter and thus seem unsuited for production of xanthate rayon unless specially treated, for example, by a mild acidic "prehydrolysis" of the wood to remove pentosans and perhaps other substances before the kraft pulping reaction.

This prehydrolysis step, which so influences the properties of the residual wood that cellulose can be dissolved in cuprammonium hydroxide or xanthating solutions,[70] is mentioned here because it may yield an aqueous sugar solution suitable for microbiological preparation of alcohol, yeast, or similar products. Prehydrolysis with water alone has been proposed for use with beech wood,[189] where the acidic environment results from the formic and acetic acids from the wood itself. Dilute solutions of other acids, such as hydrochloric, sulfuric, and phosphoric,[116,161] have been suggested for use, as well as sulfurous acid and unfermented sulfite waste liquor.[166,168] At the Johannesmuhle, Germany, plant of the Waldhof Fabrik Zellstoff, industrial-scale prehydrolysis of pine was conducted with water at 140°C for ½ to 2 hours to remove 6 to 8% of hemicellulose. By subsequent kraft pulping and bleaching, a pulp used for viscose rayon manufacture was produced. Production of

yeast from the sugars in the prehydrolysis liquors has been considered.[105,134]

### The Composition of Sulfite Waste Liquor

Calcium-base sulfite waste liquor is usually a brown, aqueous solution the density of which is not much higher than that of water. The liquor ordinarily contains some 8 to 12% dissolved solids which consist mainly of calcium lignin sulfonates and sugars, together with many other inorganic and organic substances, present in minor amounts, e.g., methanol, ethanol, acetic and formic acids, furfural, terpenes, and resins. Analytical results obtained with two calcium sulfite waste liquors[158] are given in Table 16. Other data have been published, e.g., by Baum, Bard, Salvesen, and Brabender.[20]

TABLE 16. COMPOSITION OF TWO CALCIUM SULFITE WASTE LIQUORS

| Component | Sample A | Sample B |
|---|---|---|
| | grams per liter | |
| Total solids | 115.4 | 121.6 |
| Ash | 11.9 | 20.9 |
| Calcium oxide | 6.2 | 9.6 |
| Free sulfur dioxide | 0.6 | 8.0 |
| Loosely-combined sulfur dioxide | 3.7 | 6.2 |
| Sulfate, as sulfur trioxide | 1.1 | 1.1 |
| Total sulfur | 8.9 | 15.8 |
| Methoxyl | 7.8 | 8.1 |
| Total reducing substances, as glucose | 26.8 | 20.5 |
| Fermentable sugars, as glucose | 19.1 | 12.5 |

Source: Peniston and McCarthy[158] (Reprinted by permission from the *Journal of the American Chemical Society*).

Lignin sulfonates, which are apparently propyl-phenol type polymers,[31,60,69,104,142,159] containing about one sulfonate group for each two structural units, constitute about 65% of the dissolved solids and are in solution mostly neutralized by calcium or other basic ions.

The total sulfur in sulfite waste liquors may amount to about 8% of the weight of the solids and is present in the following forms: (1) "free sulfur dioxide," i.e., sulfurous acid and bisulfite ions; (2) "loosely bound sulfur dioxide," i.e., derivatives readily hydrolyzable by dilute alkali and consisting mostly of carbohydrate and lignin bisulfite addition compounds; (3) "tightly bound sulfur

dioxide, i.e., sulfonic acids of lignins and sugars, which are hydrolyzable by heating in rather strong alkaline solution; and (4) sulfate, thiosulfate, and polythionic acids present usually in only small amounts.

The sugars in technical softwood sulfite waste liquor, which usually amount to about 20% of the total solids, are present largely as monosaccharides.[145] Approximate determination of the total concentration of these sugars can readily be made by reducing value methods.[127] In interpreting such results, it should be remembered that a mixture of sugars is being analyzed and that some reducing power seems to be associated with the lignin sulfonates[158] and carbonyl-containing minor constituents.

Table 17. COMPOSITION OF SULFITE WASTE LIQUOR SUGARS

| Sugar | 85% Western hemlock plus 15% white fir[a] % | Spruce[b] % |
|---|---|---|
| Xylose | 15 | 17.0 |
| Arabinose | 6 | |
| Mannose | 48 | 42.7 |
| Fructose | 2 | 4.0 |
| Glucose | 15 | 28.9 |
| Galactose | 10 | 4.2 |
| Galacturonic acid | –– | 3.2 |
| Unidentified | 4 | –– |
| Total | 100 | 100.0 |

[a] Average of two determinations.[145]
[b] Undesignated species.[90]

As noted previously, the individual sugars often observed in sulfite waste liquor are glucose, mannose, galactose, possibly fructose, xylose, and arabinose. The proportions of these sugars found in two different softwood sulfite waste liquors are given in Table 17. The results of Hägglund, Klingstedt, Rosenquist, and Urban[90] were obtained by classical procedures, while those of Mulvany, Agar, Peniston and McCarthy[145] were secured by chromatographic techniques applied to the sugar solution remaining after lignin sulfonates and other anionic and cationic substances had been removed by dialysis and ion-exchange resin treatments of sulfite waste liquor. Quantitative departures from these compositions are to be expected, depending both on the conditions of the sulfite

pulping process, and on the particular species and source of the wood used. Sundman,[197] employing analytical conditions controlled so as to avoid sugar interconversion, has recently indicated that fructose is not present in spruce, pine, and birch woods, in spruce holocellulose, and in paper and rayon pulps made from spruce.

The following distribution of individual sugars in a sulfite waste liquor from aspen has been reported recently by Roschier and Aaltio:[167] 71% xylose, 9.2% mannose, 4.4% arabinose, 3.6% uronic acid, 1.4% glucose, 0% galactose, and 10.4% unaccounted for, when the total reducing material was taken as 100%. The small proportion of hexoses found suggests that very mild pulping conditions were used. Similar results were obtained with birch.

These sugars may exist in sulfite waste liquor in part as bisulfite addition compounds for which some equilibrium data have been reported.[193,194]

## PROCEDURES FOR PROCESSING SULFITE WASTE LIQUORS TO YIELD ALCOHOL

Since the disclosures about 1907 of the sulfite waste liquor alcohol processes, patented by Wallin[213] and Ekström,[50] many modifications and extensions have been suggested. Some of these will now be mentioned, roughly in the order in which the process steps may be carried out.

### Collection of the Sulfite Waste Liquor

For best economy in sulfite waste liquor alcohol production, it is very important to accomplish separation of sulfite waste liquor from unbleached wood pulp with maximum recovery of liquor at minimum dilution. Best recovery of liquor, and thus of fermentation sugars, is desirable because this assures maximum alcohol yield per ton of wood processed. Dilution of sulfite waste liquor should be minimized because, without dilution, alcohol is obtained at maximum concentration in the fermented liquor and thus the cost of steam for alcohol rectification, which may represent approximately 50% of the alcohol price,[28] is reduced. Optimum liquor collection is also important to minimize evaporation steam requirements when the liquor is to be concentrated and the solids burned as a fuel,[32,181,217,220] perhaps with process chemical recovery.

The Scandinavian practice is to permit the liquor to drain

from digesters or blowpits. Bjørnstad[28] in Norway found that under certain conditions, 1,000 gal of undiluted waste liquor was drained per ton of pulp in a digester. An additional 480 to 600 gal per ton could be recovered by injecting dilute sulfite waste liquor at the bottom of a digester and removing residual liquor at the top.

In an alternative procedure,[43] the contents of a digester are blown or dumped into a blowtank equipped with agitators, undiluted waste liquor is admixed, and pulp and liquor are then delivered to a high-density vacuum thickener by which some 74% of the undiluted waste liquor may be extracted. A somewhat similar system, although based on use of water to cause gravity displacement of sulfite waste liquor from quiet pulp in a blowpit, is in use at the Puget Sound Pulp and Timber Company plant at Bellingham, Washington, and has been described in detail by Abbott.[12] Still another procedure is to employ multistage countercurrent washing, as practiced in modern kraft mills. This procedure would give best results, but it requires considerable capital outlay. The various methods have been compared by Davis.[43]

### Preparation of Sulfite Waste Liquor for Fermentation

A collected sulfite waste liquor is usually prepared for fermentation by removing fermentation inhibitors or rendering them innocuous, by adjusting the liquor temperature and acidity to optimum levels, and by adding nutrients. Much attention has been devoted to fermentation inhibitors and, while this matter is not yet wholly resolved, sufficient progress has been made to design and operate plants without substantial difficulty from this source.

The major fermentation-inhibiting factor appears to be the presence of sulfur dioxide derivatives. Removal of lignin sulfonates with ferric chloride, as proposed, e.g., by Petrov,[160] is not necessary. The antiseptic efficiency of sulfurous acid and sulfite solutions on the yeast *Saccharomyces ellipsoideus* has been studied by Rahn and Conn,[163] who reported that "the effect of $SO_2$ on yeast is due to the undissociated $H_2SO_3$ molecule of which only 0.4 mg per 100 ml prevents multiplication" and ". . . 7 mg per 100 ml kills the yeast." This important influence of undissociated sulfurous acid was previously recognized by Hägglund.[75] Thus fermentation in untreated sulfite waste liquor is inhibited or prevented because considerable sulfur dioxide is usually present and a substantial

proportion of this will exist as undissociated sulfurous acid because of the effect of the fairly high concentration of hydrogen ions in the liquor on the position of the sulfite-bisulfite-undissociated sulfurous acid equilibrium. This situation is complicated by the fact that coexisting sugars and perhaps some lignin sulfonates and other substances form bisulfite addition compounds which tend to maintain equilibrium with the other sulfur dioxide derivatives. Thus, to avoid inhibition of fermentation, it is necessary to reduce the concentration of undissociated sulfurous acid to a low value, although Gadd[71] has indicated that acetaldehyde in concentrations above about 0.15% and furfural, as well as other minor ingredients, may be also inhibitory.

With respect to the bisulfite addition compounds, Hägglund, Heiwinkel, and Bergek[88] found that these derivatives of sugars are most stable at pH 4 to pH 7 and are not fermented but can markedly retard alcoholic fermentation, presumably through hydrolysis during fermentation to yield some undissociated sulfurous acid. However, if sulfite waste liquors are neutralized to above pH 7, these bisulfite addition compounds are reportedly hydrolyzed and alcohol yields are increased.[21,88]

In more detail, Höpner[107] studied the glucose bisulfite addition compound and found an increasing degree of hydrolysis at equilibrium as the acidity decreased or the temperature increased. However, the rate of attainment of equilibrium was slow under acidic conditions but became rapid in the range of pH 6. He also studied the behavior of xylose and mannose bisulfite addition compounds and reached the conclusion that these do not cause the difficulties sometimes experienced in sulfite waste liquor neutralization, but more stable or more slowly hydrolyzed sulfurous acid derivatives, probably lignin sulfonates, are also present. By freeing sulfite waste liquor from loosely-combined sulfur dioxide and then preparing a hydrazone, Adler[14] secured some evidence indicating that lignin sulfonic acids in fresh sulfite waste liquor are present in part as bisulfite addition compounds. However, he concluded that in rayon pulp sulfite waste liquors, the major part of the loosely combined sulfurous acid is joined with such volatile aldehydes as formaldehyde and methylglyoxal, while in paper-pulp liquors, a considerable part is attached to nonvolatile carbonyl compounds. Sundman's work[192] has indicated that fresh sulfite waste liquor contains substantial proportions of sugar bisulfite addition com-

pounds, but when this liquor has been freed from gas and neutralized to pH 5.5, these compounds dissociate almost completely so that only 10% of the loosely combined sulfur dioxide may originate from bisulfite addition compounds of sugars whereas 90% is bound to other compounds, such as lignin sulfonic acids. Thus while a predictable equilibrium is speedily reached on adjustment of the acidity of solutions of inorganic bisulfites, neither the equilibrium nor the kinetic picture is as yet entirely clear with respect to the behavior of the various organic bisulfite addition compounds.

In the Ekström process,[40] sulfite waste liquor was prepared for fermentation by neutralizing the liquor with limestone and then permitting suspended or precipitated solids to settle. In this and later similar neutralization processes, the effectiveness of the treatment is largely attributable to the reduction in undissociated sulfurous acid by conversion to apparently nontoxic inorganic salts, which, in some cases, are soluble only to a limited extent. During neutralization, care must be exercised to avoid fermentable sugar destruction by local extreme alkalinity.[35]

Aeration has also been used[56] to remove some of the sulfur dioxide into the gas phase while oxidizing another part to sulfate which has no major effect on fermentation. To accelerate air oxidation to sulfate, it has been suggested to add to sulfite waste liquor such catalysts as manganese or chromium[56] or cobalt[18] ions, or else a "peroxide material."[140] For more complete removal of inhibitors by aeration, the addition of mineral acid to sulfite waste liquor has been found advantageous by Smart,[190] Marchand,[140] and others.[15] Addition of mineral acid, together with boiling or partial concentration of sulfite waste liquor, has been considered by Tartar[199] and others.[66,131,178] More recently, it has been found possible and industrially economical to remove sulfur dioxide and other volatile inhibitors continuously and without acid addition by using a steam-stripping process[133] at elevated temperature in a multistage column. A considerable amount of sulfur dioxide is recovered for reuse and, simultaneously, the liquor can be maintained sterile and sufficiently freed from inhibitors so that, after cooling and often without neutralization, it is readily fermentable or can be used for yeast propagation. However, the report of Walker and Morgan[212] suggests that the effectiveness of steam stripping may vary with the sulfite waste liquor being treated.

To complete preparation of sulfite waste liquor for fermentation, acidity and temperature, must be adjusted and nutrients added. Limestone[55] and/or lime[49] may be used for neutralization but Løschbrandt and Troye[132] found that the acidity during fermentation had much less influence on alcohol yield than the concentration of bisulfite ion in the liquor. Wurz and Warscha[218] investigated the effects of neutralizing both paper- and rayon-pulp liquors at temperatures from 20° to 130°C and concluded that highest alcohol yields were obtained from those liquors which had been neutralized to pH 5.3 at 95° to 100°C. Industrial neutralization practice will be discussed later. For cooling, surface or vacuum equipment may be used. Nutrient requirements are small and usually may be satisfied by ammonia or urea addition.

### Fermentation of Sulfite Waste Liquor

Since many excellent sources are available for theoretical and practical information on alcoholic fermentation, in general, and much of this information is given in Chapters 2 and 3, consideration of sulfite waste liquor fermentation will be restricted to problems and developments uniquely associated with employment of this substrate. Extensive studies in this field have been conducted by Hägglund.[75,76,84,85,86,87,91,92,93,94]

During fermentation, sulfur dioxide derivatives seem to act not only as inhibitors but also as agents which give rise to formation of increased amounts of acetaldehyde.[79,198] (See also Chapter 8.) Ordinarily, this aldehyde turns up as methyl or ethyl acetals in the "heads" fraction from sulfite waste liquor alcohol-distillation systems and investigators have considered its microbiological conversion to ethyl alcohol by means of recycling this "heads" fraction to the fermenting liquor.[72,195,221]

The rate of fermentation of sulfite waste liquor sugars was slow by the early processes due, in part, to the presence of inhibitors. Thus attempts were made to develop strains of yeast so acclimated to the liquor that more rapid fermentation and better alcohol yields resulted.[51,118,186] The acclimatization of various microorganisms to wood hydrolyzate solutions has recently been studied by Johnson and Harris.[112] Removal of carbon dioxide has been suggested as a means of speeding up fermentation.[148]

However, another factor, influencing the rate of fermentation,

is the concentration of yeast in the liquor being fermented. Under Ekström's conditions,[40] fermentation was initiated by adding freshly prepared sulfite waste liquor to a fermentor containing a "bottoms inoculum," consisting of some previously fermented liquor, together with suspended yeast. During fermentation, this yeast increased to about ten times the amount originally added. Similarly it has been proposed[147] to maintain a continuous flow of the fermenting liquor through a fermentation vat and to conduct part of this liquor back to the inlet of the vat where it can serve as inoculum. Continuous fermentation may also be obtained by the fixed-yeast method, passing the liquor at a suitable rate through one or more vessels each of which contains masses of yeast fixed on a carrier or packing material, such as kieselguhr[165] or chips.

Rosten[171,176] has pointed out that fermentation procedures, employing the bottoms inoculum method have the undesirable economic feature that most of the yeast present during each fermentation is removed in the fermented liquor and goes to the distillation system where it is killed and passes to waste. He has emphasized the advantages associated with the "reuse of the yeast process" of the Usines de Melle[29,30] which is reported to be employed in at least one hundred distilleries in Europe. It was first used with sulfite waste liquor at the Attisholz plant in Switzerland. In 1939, five plants in Europe were operating on this process, using sulfite waste liquor (Cosel, Wagen, Oberleschen, Heidenau, Attisholz). Essentially, the process comprises the centrifugal separation of a yeast cream from the fermented liquor and the reuse of this recovered yeast as an inoculum for batch fermentation of new liquor. Advantages cited for the method are: (1) The alcohol yield is improved by about 15%, because the relatively high yeast concentration present at inception of fermentation does not greatly increase and thus sugar otherwise consumed in yeast growth is converted to alcohol; (2) yeast seed preparations are unnecessary and the yeast culture functions most effectively because it is acclimatized to the environment to the maximum degree and may utilize sugars which are not usually fermented; (3) size and thus capital costs of equipment for fermentation may be decreased because faster fermentation results from well-acclimated yeast present at relatively high initial concentration; (4) contamination problems are minimized because undesirable organisms are often lighter than yeast and are not separated with the yeast in the

centrifuges, but remain with the fermented liquor and go to the beer stills where they are killed.

The concentration at which alcohol is obtained in fermented sulfite waste liquor is a factor of primary economic concern in alcohol production. This occurs because the costs of recovery and concentration of alcohol to the usual industrial strength increase rapidly as the alcohol concentration in the fermented liquor decreases. One way of obtaining alcohol at relatively high concentration is to reuse 40 to 50% of undiluted sulfite waste liquor from a preceding cook for a succeeding one as recently proposed and investigated.[95] Another possibility is to concentrate sulfite waste liquor prior to fermentation, and this has been suggested periodically since 1910.[15,49,174,202] Concentration by evaporation of calcium sulfite waste liquor has presented some difficulty because of deposition on heater surfaces of calcium sulfate scale,[173,211] but recent reports indicate that these difficulties are being eliminated.[36,46,122,169] Concentration by evaporation of magnesium sulfite waste liquor is now being conducted on an industrial scale, and Tomlinson[201,202] has patented fermentation of this type of liquor at up to about 30% total solids. The rates and yields of fermentation of calcium, magnesium, and ammonium sulfite waste liquors under various conditions of total solids concentration and of acidity have been studied.[22,34,58,68,174]

Another way to secure higher concentrations of alcohol in fermented sulfite waste liquor is to add to the liquor, prior to fermentation, substances which contain or yield fermentable sugars, such as glucose from hydrolysis of cellulose,[52] or starch,[55] or molasses.[64] Molasses was added at the Mechanicsville, New York, plant. Sugar sorption by yeast, followed by separation of the yeast with later fermentation in the presence of minimum water, has also been suggested.[47]

Many other microbiological conversions of sulfite waste liquor sugars have been studied,[125] for example, those yielding butyl alcohol, acetic, propionic, and butyric acids,[42,214] lactic acid,[126] antibacterial substances by *Penicillium notatum*,[129] fuel gas,[215] and yeast. (See Chapter 10.)

## Alcohol Distillation and Purification

After the steps of sulfite waste liquor fermentation and yeast removal have been completed, the alcohol must be recovered, con-

centrated, and separated from impurities. It may be desirable to scrub the fermented liquor with air[17] to remove carbon dioxide which would cause foaming in the stills. This is practiced in the A. B. Tegefors plant at Järpen in Sweden.[10]

In beer stills, the alcohol is removed from the liquor in the first distillation step. The residual liquor leaves the system as bottoms, while dilute ethyl alcohol, along with methyl alcohol and other volatile impurities, passes off as overhead vapors. To reduce to a minimum the steam necessary for recovery of alcohol from the dilute solutions of sulfite waste liquor alcohol, the Othmer vapor-reuse[153] or solvent-extraction schemes[154] might be considered.

The alcohol stream from the beer stills may be passed to a rectifying column from which the concentrated ethanol is recovered as tops. Fusel oil side streams may be removed, containing propyl, butyl, isobutyl, amyl, hexyl, and heptyl alcohols, with amyl and hexyl alcohols predominating.[188] Reports have been made of the presence of such other substances as borneol,[54] limonene,[103] camphene, fenchyl alcohol, guaiacol, and perhaps azulene,[117] and 3-methoxy-4-hydroxypropylbenzene.[110] The removal of terpenes from alcohol by distillation has recently been studied by Hähnel.[96] Treatment of alcoholic vapors with a sodium hydroxide solution has been proposed to remove volatile acids possibly present.[57]

The overhead stream from the rectifying column may be introduced into a purifying column which separates a light "methanol heads" fraction from the bottoms ethanol product. This methanol may originate from methyl esters in polyuronide hemicelluloses and seems to be formed in part during sulfite pulping and in smaller proportion during fermentation.[82] It is interesting to note that in 1910 it was thought not practicable to separate methanol and ethanol[25] and in 1914 this separation was considered only theoretically possible.[2] Modern distillation systems allow[26,149,150] complete separation of these two alcohols. The "heads" fraction is largely a mixture of methyl and ethyl alcohols, acetaldehyde, and acetals. Hydrolysis of acetals by an acid salt followed by separation of components has been suggested[207] as a means of obtaining pure ethanol, methanol, and acetaldehyde. Under some circumstances, hydrogen sulfide appears and may combine with acetaldehyde to yield thioacetaldehydes.[74] It should be emphasized that through modern technology, sulfite waste liquor alcohol can

now readily be produced equal in quality to the best grades from other sources.

Dehydration to produce anhydrous alcohol useful as motor fuel has been of special interest in Europe and drying processes have been suggested based on use of quicklime,[179] benzene,[115] potassium acetate,[44,180] trichloroethylene ("Drawinol"),[45] and calcium sulfate.[152]

### Analytical Methods

Sulfite waste liquor ingredients can be determined, using methods recommended by Partansky and Benson,[156] and others more recently considered by Yorston.[219] A procedure for the evaluation of biochemical oxygen demand of sulfite waste liquor has been suggested by Tyler and Gunter.[206]

Reducing or fermentable sugars can be estimated by methods recommended by Tappi[156] or by standard Swedish[38] or German procedures.[183] Menzinsky[144] has suggested a rapid method based on absorption of fermentable sugars (mannose, fructose, glucose, galactose) from sulfite waste liquor solutions by the yeasts *Saccharomyces cerevisiae* or *S. fragilis.* Individual sugars in sulfite waste liquor can be calculated, for example, from differences in dissociation of their bisulfite addition compounds,[193,194] or by column[145] or paper[65,101] chromatography.

A Swedish method is available[39] for determination of yield of alcohol obtainable from sulfite waste liquor by fermentation. Sulfite waste liquor alcohol quality is evaluated by the usual methods.

## MODERN SULFITE WASTE LIQUOR ALCOHOL PLANTS

For two Swedish alcohol plants completed in 1942, a description of process steps with flow sheet and illustrations has been published.[10] The installations were based on the system of Tesch A. B. of Stockholm, involving the steps of neutralization with lime and of continuous fermentation. The plant of the Forss A. B. was located at Köpmanholmen and the plant of the A. B. Tegefors, at Järpen. The production capacity of each of these plants is around 400,000 gal per year.

A description of the equipment of a typical German sulfite alcohol plant, the sequence of operations, the distillation of the

liquor, and the utilization of the residues was published in 1949.[114]

In North America, production of ethyl alcohol from sulfite waste liquor was undertaken at Thorold, Ontario, and at Bellingham, Washington, during World War II to aid in meeting wartime needs for butadiene and styrene for synthetic rubber, explosives, solvents, and other chemicals. A third plant was established at Gatineau, Quebec, shortly after the war. Although some features of the design of these plants may have resulted from wartime expedients rather than from optimum requirements, they will be described in some detail as representative of most recent practice.

## The Ontario Paper Company Plant

This plant, the first recent major installation in North America, was constructed at Thorold, Ontario, in 1942-3 by the Ontario Paper Company Limited. It has been described by Sankey and Rosten[170,175,176] and by Joseph[113] and others.[1,37] Chrome-nickel steel, containing a small proportion of molybdenum was used for all equipment exposed to severe corrosion conditions.

To recover a maximum volume of sulfite waste liquor at minimum dilution, stainless steel and rubber-covered pulp washing equipment were desired, but these materials were not available at the time of construction. Thus sulfite waste liquor was recovered by blowing digesters into dry pits and controlling drainage through the perforated floors to obtain a maximum degree of separation of liquor and fiber, using minimum wash water in the time allowable before preparation of the pit for the next digester blow. By this procedure about 60% of the total sulfite waste liquor discharged from the digesters is recovered at about 80% of digester concentration. This amounts to 6.5 tons of liquor per ton of pulp produced.

As initially constructed, the sulfite waste liquor was pumped over inclined screens to remove residual fiber and then passed to storage in a 100,000-gal tank. Shortly after the war, a countercurrent steam-stripping tower was installed before the screens.[209] From storage, the liquor is cooled to fermentation temperature (90°F) in water heat exchangers and is then passed to one of three neutralizing tanks where lime is added to a pH of 5.5 to 6.0. After settling, the sludge is discharged to the sewer and the clear liquor is pumped to the fermentors. Nutrient salts are introduced at the

pump suction in passage to the fermentors or directly in the fermentors.

Fermentation is conducted batchwise with the yeast reuse process of Les Usines de Melle.[29,30] About 40,000 gal of liquor is circulated between a pair of fermentor tanks with yeast slurry added from a previous batch. Fermentation is conducted for 12 to 14 hours following which a 1- to 2-hour sludge settling period is allowed. The liquor is then decanted to centrifugal yeast separators which discharge a yeast slurry at about 15% concentration. This is stored in 2,100-gal tanks for use in subsequent fermentations.

The clear liquor from the yeast separators is discharged to a beer well for feed to the stills. Two beer stills are operated with parallel flow. They discharge a 10% alcohol vapor which is partially condensed and fed to the rectifying column. The top fraction from the rectifying column is concentrated ethanol with some aldehydes, ketones, and acetals, and also methanol. It is refractionated in a purifying column to yield ethanol and an impure methanol for use as industrial solvent. Table 18 shows operating data given by Joseph[113] for the Ontario Paper Company plant for two months, one in 1943 shortly after construction and the other in 1946 after modifications and improvements had been made.

TABLE 18. DAILY AVERAGE DATA ON SULFITE WASTE LIQUOR ALCOHOL PRODUCTION AT THE PLANT OF ONTARIO PAPER CO., LTD.

| | October 1943 | June 1946 |
|---|---|---|
| *Sulfite waste liquor* | | |
| Received from mills, Imp. gal per day | 210,600 | 227,953 |
| Recovered, Imp. gal per ton of pulp | 1,302 | 1,140 |
| Specific gravity | 1.046 | 1.049 |
| *Fermentable sugars* | | |
| Processed, lb per day | 28,111 | 32,852 |
| Concentration in liquor, g per l | 1.39 | 1.65 |
| *Chemicals used per 100 gal alcohol* | | |
| Nutrient salts, lb | 6.1 | 2.9 |
| Lime, lb | 431.2 | 226.0 |
| Sulfuric acid, lb | 14.1 | 0.0 |
| *Ethyl alcohol* | | |
| Produced, Imp. gal per day | 1,450.9 | 1,805.0 |
| Strength, over proof[a] | 68.8 | 68.7 |

[a] 68.8 over proof corresponds to 96.3% ethyl alcohol by volume.
Source: Sankey and Rosten,[176] and Joseph.[113]

For process control,[113] sugar determinations are carried out by yeast fermentation and measurement of carbon dioxide evolved. Alcohol is estimated by a Raleigh-type laboratory distillation followed by measurements of density or refractive index of the distillate. The liquor going through the alcohol plant is reduced about 43% in b.o.d. Alcohol product analyses, exemplified by the data given in Table 19, met or exceeded U. S. Army Specifications No. 1. Sulfur contained in Ontario Paper Company alcohol was found to be between 1.4 and 2.1 ppm which is close to the limit of the determination.

TABLE 19. CHARACTERISTICS OF SULFITE WASTE LIQUOR ALCOHOL FROM THE ONTARIO PAPER COMPANY PLANT IN AUGUST 1944

| | |
|---|---|
| *Concentration, Canadian over proof*[a] | 68.7 |
| Residue on evaporation,% | 0.0009 |
| Residual acidity,[b]% | 0.0009 |
| Permanganate time, minutes | 29 |
| Aldehydes, ppm | 25 |
| Fusel oil, ppm | 12 |
| Methyl alcohol | Nil[c] |

[a] 68.8 Canadian over proof corresponds to 96.3% ethyl alcohol by volume.
[b] Sankey and Rosten[176] report only 1.4 to 2.1 ppm of total sulfur found by analysis of the sulfite waste liquor alcohol ordinarily manufactured.
[c] "Each day below 0.1% which is minimum determinable quantity."[113]
Source: From Joseph[113] as averages over the month of daily evaluations.

Alcohol yields cannot be directly estimated from the data given in Table 18, since a portion of the sulfite liquor is used for yeast production in a separate plant. However, Sankey and Rosen[176] calculated from 1943 operations a yield of 13.3 Imperial gal O.P. (Canadian) per ton of pulp, after application of the proper factors. This corresponds to 16.6 U. S. gals of 96% alcohol per ton of pulp.

## The Puget Sound Pulp and Timber Company Plant

One major plant was built in the United States during World War II for production of alcohol from sulfite waste liquor. The Puget Sound Pulp and Timber Company of Bellingham, Washington, as agents for the Defense Plant Corporation, undertook construction and operation of this plant only after considerable investigation to work out a process satisfactory for this particular sulfite mill. This process, which uses simplified plant operation

and reduced production costs, differs from published European practice in the following respects: continuous flow throughout, recovery of sulfur dioxide by steam stripping, fermentation under relatively acidic conditions, and flash cooling. About 9,000 gal of 190°-proof ethyl alcohol are produced a day, which is equivalent to a yield of about 22 U. S. gal of 95% ethanol per ton of pulp. Conditions and procedures for pulp production were in no way changed to accommodate the alcohol plant and the quality of the sulfite waste liquor has remained very uniform in composition and satisfactory for processing.

This plant has been excellently described by E. O. Ericsson[62,63] and others,[3] whose reports will be closely paraphrased here under the following five headings: (1) recovery and collection of the liquor, (2) preparation of liquor for fermentation, (3) addition of yeast and fermentation, (4) separation of the yeast for reuse, and (5) distillation. A generalized flow sheet is given in Figure 21.

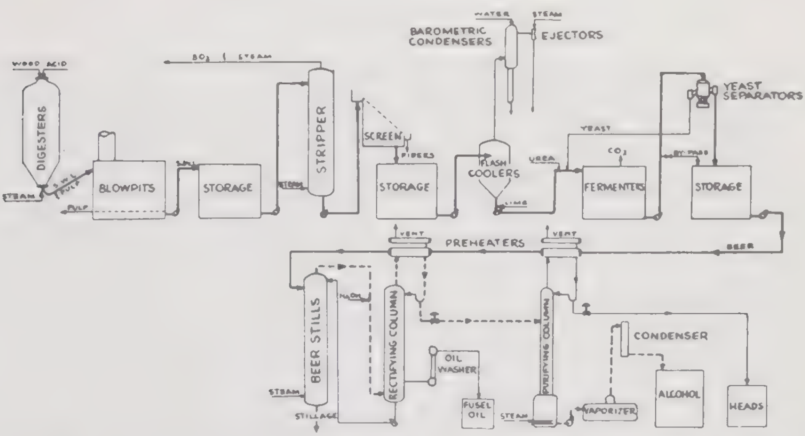



FIGURE 21. *Generalized Flow Diagram for Sulfite Waste Liquor Alcohol Plant.* Reprinted by permission from a paper by E. O. Ericsson, *Chem. Eng. Progress,* **43**, 165 (1947).

## Recovery and Collection of the Liquor

While countercurrent washers were desirable to provide maximum liquor recovery with minimum dilution, existing equipment was used for economical reasons. This consisted of round wooden "blowtanks," 36 ft in diameter and equipped with perforated stainless-steel bottoms. These blowtanks are filled with sulfite

waste liquor to a depth of about 4 ft and then the pulp and liquor from a digester is blown into and allowed to settle in the tank containing the liquor so that uniform distribution of pulp over the tank bottom is obtained. Under these conditions, the hot sulfite waste liquor drains from the pulp and is finally displaced with water.[12] When water breaks through the pulp bed, automatic controls detect the temperature drop and act to divert the dilute waste stream from the accepted stream which amounts to more than 2,000 gal of sulfite waste liquor per ton of pulp. The liquor is at 92° to 93°C and has a specific gravity of about 1.05 and a pH of about 2.2. It contains, per liter, 120 g total solids, 2 g free sulfur dioxide, 4 g loosely combined sulfur dioxide, and 19 g fermentable sugars.

PREPARATION OF THE LIQUOR FOR FERMENTATION

Removal of sulfur dioxide and other toxic substances is accomplished by passing the liquor through a twenty-plate stainless-steel column (about 8 ft in diameter and 45 ft tall) while steam flows countercurrently up the column to accomplish complete

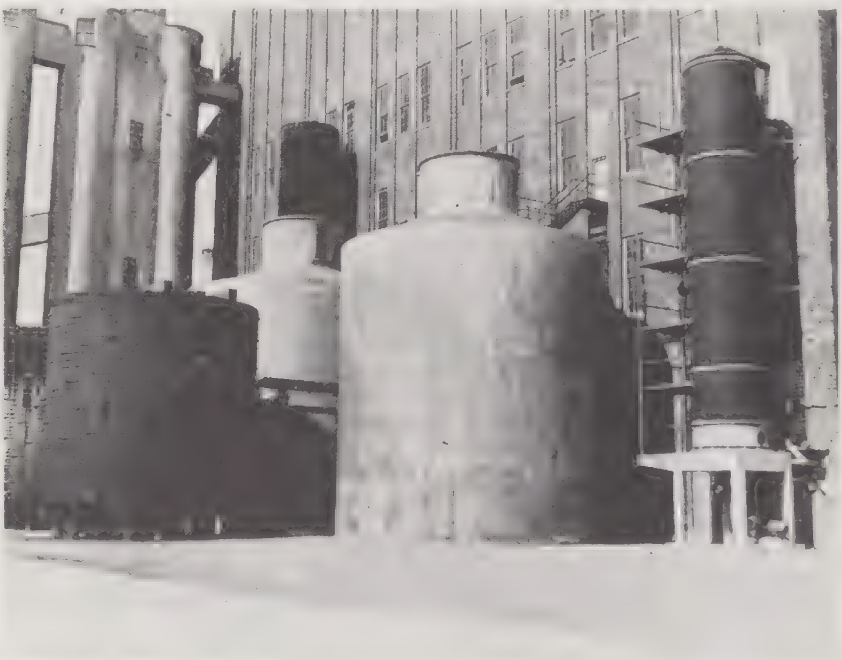


FIGURE 22. *Liquor Steam Stripper (on right) in a Pulp-Mill Installation* (Courtesy—Puget Sound Pulp and Timber Co., Bellingham, Wash.)

removal of the free sulfur dioxide and partial removal of the loosely combined sulfur dioxide. Sulfur dioxide and steam from the top of the column are injected into the digester cooking acid and are thus reused. By this steam stripping, about 20 lb of sulfur are recovered per ton and this offsets the cost of steam requirements. About ½ lb of steam is generally used per gallon of liquor feed and the pH of the stripped liquor is 3.8 to 4.2.

Although the acidity of a sulfite waste liquor can be sufficiently reduced by steam stripping to make further treating unnecessary, most economical operation is obtained by addition of about 3 lb of lime per 1,000 gal of sulfite waste liquor treated. This neutralization step is conducted in the alcohol-plant building by adding a 10% slurry of lime in water to yield about pH 4.5. After neutralization, the liquor is cooled from about 105° to 30°C, using two flash tanks, each operating with a barometric condenser for producing a high vacuum. Under these conditions, the liquor is concentrated about 12%. The cooled liquor is filtered to remove pulp fibers and then pumped to storage tanks.

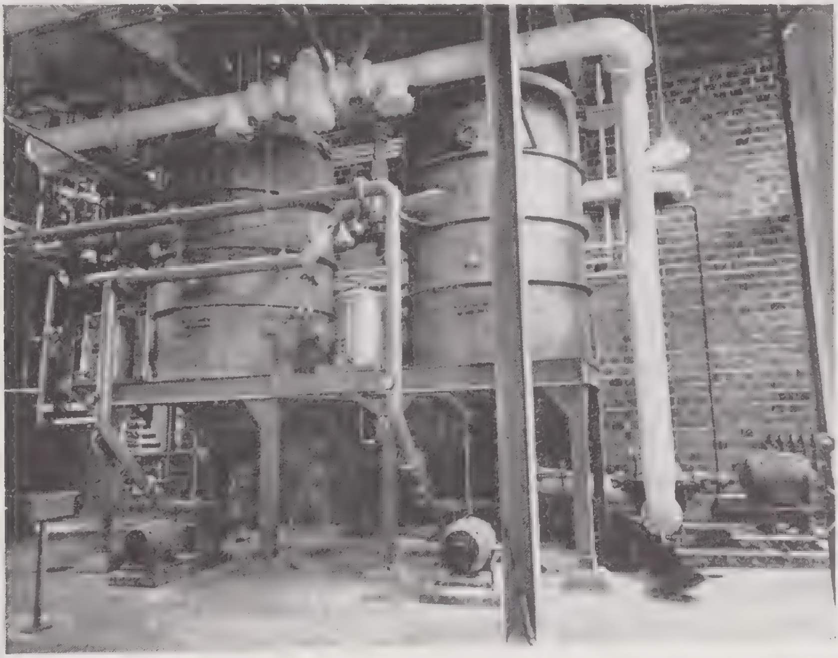


FIGURE 23. *Flash Coolers and Pumps in the Neutralization Department* (Courtesy—Puget Sound Pulp and Timber Co., Bellingham, Wash.)

ADDITION OF YEAST AND FERMENTATION

Fermentation is conducted with the yeast *Saccharomyces cerevisiae,* using eight interconnected fermentors of 80,000-gal capacity each. The plant was started up by adding a quantity of yeast obtained from a commercial source and operations were continued with yeast which grew up in the fermentors simultaneously with alcohol production and amounted to about 25 tons in all. Fermentors are operated at 30° to 35°C, pH 4.3 to 4.7, containing about 0.5 to 1.0% by volume of suspended yeast, with small additions of nitrogen as ammonium hydroxide. Liquor from storage is mixed with measured amounts of ammonium hydroxide and yeast and then enters the first fermentor from which it overflows to the second, etc., through the eighth and last fermentor. From 70 to 80% of the fermentable sugars is converted in the first two fermentors, and about 95% in the series of eight. Fermentation time has varied from 12 to 20 hours.

FIGURE. 24. *Fermentor Tanks, Piping and Agitators from the Lower Walkway* (Courtesy—Puget Sound Pulp and Timber Co., Bellingham, Wash.)

Control of fermentation consists of regular measurement of the sugar concentration in the liquor entering and leaving the fermentors and of the alcohol content of the fermented liquor. Yeast is examined daily for viability and cell count.

SEPARATION OF THE YEAST FOR REUSE

For yeast recovery and reuse, the liquor from the last fermentor, containing about 1% yeast by volume, is fed through stainless-steel de Laval centrifuges to yield one stream containing about 15% yeast by volume which is returned to the fermentors, and a second stream with about 0.02% yeast which flows through storage tanks to distillation columns. Part of the fermented liquor is by-passed around the centrifuges and thus some yeast is not recycled to avoid undesirable accumulations which might develop if the system were a completely closed cycle.

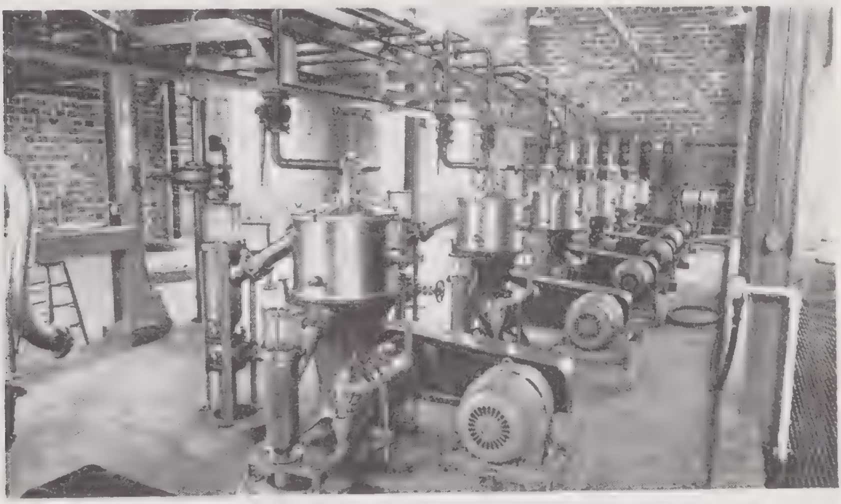


FIGURE 25. *Battery of Beer Centrifuges for Recovery of Yeast for Reuse* (Courtesy—Puget Sound Pulp and Timber Co., Bellingham, Wash.)

DISTILLATION

After separation of yeast, the fermented liquor is passed through beer stills by means of which alcohol is removed almost completely from the fermented sulfite waste liquor which leaves the bottom of the column as process effluent. These two columns were installed to permit operation in parallel with one at higher pressure than the other, so that the temperature of the vapor tops is sufficiently high to permit the enthalpy of vapors to be reused to generate low-pressure steam for operation of the other stills according to a patented process of the Vulcan Copper and Supply

Company, designers and manufacturers of this steam-saving equipment.

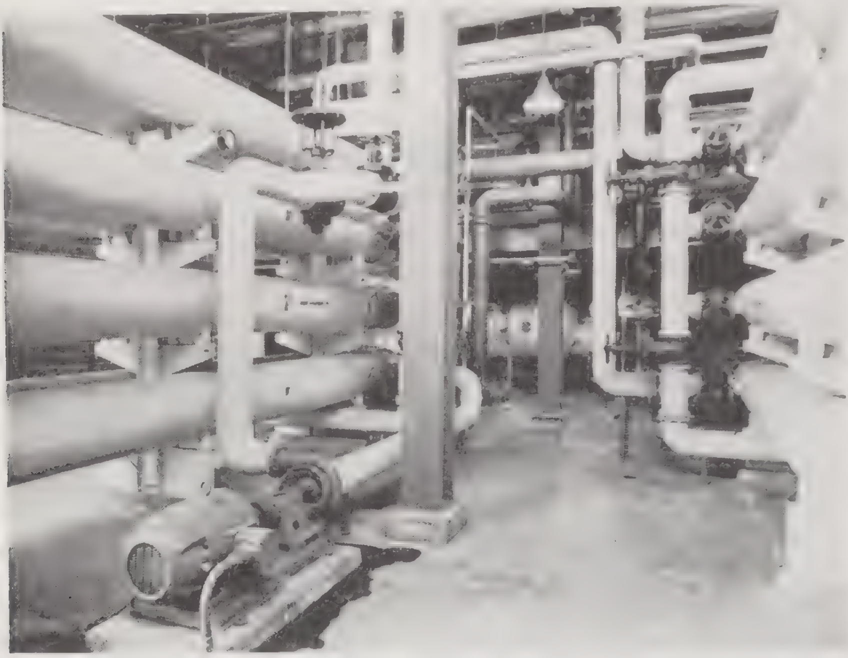


FIGURE 26. *Beer and Stillage Heat Exchangers, Showing Recirculating-Pump Controls and Piping, with a Kettle Still in the Background* (Courtesy—Puget Sound Pulp and Timber Co., Bellingham, Wash.)

Vapors from these initial distillation units containing about 8% alcohol by volume are scrubbed with sodium hydroxide solution to neutralize and remove acids. The vapors then feed to the base of the rectifying column from which alcohol is drawn as tops at about 192° proof. Fusel oil, amounting to about 10 gal a day and comprising principally the higher boiling alcohols, accumulates on intermediate plates and is withdrawn to an oil washer where it is washed free of alcohol and then deposited into storage. Alcohol from the rectifying column is then fed to the purifying column from which low-boiling methanol and aldehyde contaminants, amounting to about 175 gal a day, are withdrawn as "heads." The highly purified 192° proof industrial alcohol is removed from the bottom of the column. This alcohol is deposited in a bonded warehouse from which it is withdrawn and pumped into tank cars for rail shipment.

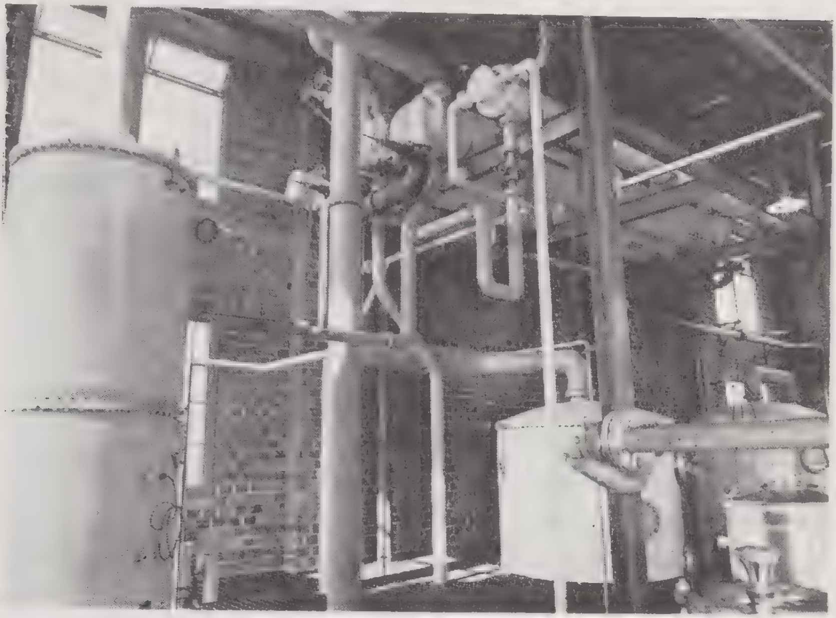


FIGURE 27. *Tops of Purifying, Atmospheric and Pressure Beer Stills, with Beer Preheaters and Vent Condensers Above* (Courtesy—Puget Sound Pulp and Timber Co., Bellingham, Wash.)

**The Commercial Alcohols Limited Plant**

This sulfite waste liquor plant,[6,8] the newest in North America, was constructed in 1948-49 at Gatineau, Quebec, on a property adjacent to mills of the Canadian International Paper Company. The plant, which cost about $3,000,000, is owned and operated by Commercial Alcohols Limited. It was engineered by the Vickers-Vulcan Process Engineering Company Limited of Montreal. Design production is 9,000 U. S. gal of 95% alcohol a day from 900,000 gal of sulfite waste liquor.

The sulfite waste liquor is obtained from ten digesters in the Canadian International Paper Company plant which has a daily capacity of 365 tons of sulfite pulp. Liquor recovery is accomplished in two parallel sets of three-stage rotary washers. These are of Improved Paper Machinery Company design, made of stainless steel with rubber-covered drums, each 8 ft in diameter by 10 ft face. The first two stages in each set are used for liquor separation and the third, for pulp washing.

The recovered liquor is pumped to the alcohol plant where, while remaining hot, it flows through five 120,000-gal storage tanks of British Columbia fir. The liquor is then cooled to 82°F by passing it through thirty-six heat exchangers constructed with stain less-steel tubes and steel shells, using cold, treated water from the paper-mill boiler house as the cooling agent and returning this heated water to the boiler plant. The cooled liquor is next neutralized to pH 5 with a slurry of hydrated lime which is mixed continuously in appropriate quantity with sulfite waste liquor in a stainless-steel reaction vessel. Since over-all ecenomics of the Gatineau installation are thought not to justify steam stripping of sulfite waste liquor for sulfur dioxide recovery, more lime is used than in the Puget Sound Pulp and Timber Company plant.

The neutralized liquor is pumped to seven fermentation tanks, each of 120,000-gal capacity and made of British Columbia fir. The fermentation is continuous and the liquor flows in series from one tank to another with a stainless-steel propeller agitator operating in each tank. From the last fermentors, the liquor passes through eight de Laval separators from which a portion of the yeast is recovered and sent back to the first fermentor. From these separators, a beer, containing about 1% ethyl alcohol by volume, flows to a beer well.

The beer from the yeast separators is preheated in stainless-steel heat exchangers and then passes to two stainless-steel beer stills. The first still, 10.5 ft in diameter by 52 ft in height, is operated at 3 psi gage pressure, and the second still, 8.5 ft in diameter by 53 ft, under vacuum. Alcohol vapors and steam from the pressure still are used in a stainless-steel steam generator to provide heat for the vacuum still. Condensed vapors from both stills are combined and sent to a purification column. This arrangement differs from that used by the Ontario Paper Company and the Puget Sound Pulp and Timber Company. The purification column, constructed completely of Type 316 stainless steel, is operated under 40 psi gage pressure to yield a light fraction of impurities, with the dilute alcohol product as bottoms. This alcohol passes through an 8.5 ft diameter stainless-steel-clad rectifying column, operating under vacuum and heated by low-pressure steam from the steam generator. The product is 95% alcohol of a grade suitable for use as antifreeze, shellac solvent, and other industrial solvent and for chemical purposes. For a best-grade product,

"Cologne Spirits," suitable for cosmetics and pharmaceutical products, the 95% alcohol is redistilled in two copper vacuum stills 52 in. and 54 in. in diameter by 54 ft high.

The entire process is automatically controlled and recorded with regard to flow, pressure, temperature, and liquor levels, so that only three operators are required per shift. Through extensive use of stainless steel, maintenance costs due to corrosion are minimized.

By-product carbon dioxide is utilized for the manufacture of a magnesium insulating material by a patented process in which a slurry of magnesium oxide or hydroxide is carbonated with gas from the fermentors and then mixed with 10% asbestos fiber. The product is molded in a machine, developed by the Johns Mansville Corporation, into 85% magnesia slabs and pipe coverings. Rated capacity of the installation is 3,000,000 board feet per year.

## ECONOMIC ASPECTS

Since industrial production of sulfite waste liquor alcohol was first initiated around 1909, many new plants have been built, especially during war periods. Most of these are located in Sweden, Finland, Norway, and Germany. The annual production in each of these countries from 1909 to 1948, along with the years during which the new alcohol plants were brought into production, has been tabulated by Lassenius.[124] In 1945, it was reported[109] that the annual sulfite waste liquor alcohol production capacity in Sweden amounted to about 31,600,000 gal of 95% alcohol and was conducted in thirty-three plants. By 1948, there were thirty-four plants in operation, with capacity to process some 80% of the sulfite waste liquor produced in that country.[144] In Germany, most sulfite pulp mills operating with spruce wood utilized sulfite waste liquor to produce alcohol which amounted to about 6,500,000 gal per year.[130,135] The outlook for alcohol manufacturers in Finland has been reviewed.[120]

In North America, the three sulfite waste liquor alcohol plants can produce annually about 7,000,000 gal of 95% alcohol. This capacity can be compared with a possible United States and Canadian production of at least 90,000,000 gal of 95% alcohol a year, assuming that all sulfite pulp mills produced alcohol, that the yield would be about 20 gal per ton of pulp, and considering

that production of sulfite pulp in 1949 was 2,537,084 tons in the United States, 1,989,000 tons in Canada, or a total of 4,526,084 tons.[7]

Two of the North American sulfite waste liquor alcohol plants were constructed to supply alcohol needed during World War II. The other plant, at Gatineau, Quebec, however, was built after the war emergency by private interests and thus seems to manifest that responsible persons believe that sulfite waste liquor alcohol could be produced, in this Canadian plant with associated individual conditions, to compete satisfactorily with alcohol entering the market from other sources.

Recent detailed costs of sulfite waste liquor alcohol production are not available. However, it is known that to process a liquor so dilute in fermentable sugars, a heavy capital investment is necessary for plants. This amounts to about $1.00 per U. S. gallon capacity per year, which suggests an amortization charge of about 3 to 5 cents per gallon if the plant is amortized in 20 years. The relative values of some manufacturing-cost items can be gained from Table 20, showing some German figures recorded by Hill and Campbell,[105] who acted as a British Intelligence Objectives Subcommittee. These are given as the 1942 manufacturing requirements and costs for the Zellstoff Fabrik Waldhof plant at Kelheim, Bavaria, in which about 2,000 gal of absolute alcohol was produced a day from spruce paper-pulp sulfite waste liquor. Swedish practice was used and the hot liquor was neutralized, aerated, allowed to settle, and the sludge removed, fermented in a 48-hour cycle with only limited yeast recovery, and distilled and rectified by the Drawinol process for use as motor fuel. Considerable decrease in several of these cost items would probably result by introducing steam stripping to recover sulfur dioxide and to reduce neutralization expense, reuse of the yeast in fermentation to conserve nutrients and improve alcohol yields, and reuse of heat in multieffect distillation systems to lower steam costs. However, such installations would increase capital investment and thus amortization costs.

Under North American conditions, costs of 1 U. S. gallon of 95% alcohol from sulfite waste liquor have been estimated as follows: about 12¢ for manufacturing cost by M. M. Rosten in 1943;[37] about 10¢ for manufacturing cost by G. H. Tomlinson in 1948;[200] and not less than 20¢ for production cost by E. Ekholm in 1951.[48] The cost figures given by Ekholm refer to sulfite waste liquor alcohol produced in plants built under 1951 conditions

TABLE 20. REPORTED 1942 REQUIREMENTS AND COSTS OF MANUFACTURING ALCOHOL FROM SULFITE WASTE LIQUOR AT THE ZELLSTOFF FABRIK WALDHOF PLANT

| Item | Unit cost[a] RM per 100 kg | Requirement per 100 l alcohol Amount, kg | Cost, RM |
|---|---|---|---|
| Materials: | | | |
| Wood sugar | 165.1 | 1.45 | 2.394 |
| Lime from sugar mills | 408 | 0.52 | 2.122 |
| Ammonium sulfate | 2.04 | 16.05 | 0.327 |
| Soda | 1.0 | 10.05 | 0.101 |
| Drawinol | 0.120 | 57.50 | 0.069 |
| Potassium hydroxide | 0.017 | 97.50 | 0.017 |
| Stabilizer | 0.003 | 690.0 | 0.021 |
| Total materials | | | 5.05 |
| Operations: | | | |
| Neutralization | | | 2.11 |
| Fermentation | | | 2.35 |
| Distillation | | | 11.50 |
| Rectification to absolute alcohol | | | 1.83 |
| Total operations | | | 17.79 |
| Total manufacturing costs[a] including labor and supervision but not amortization | | | 22.84 |

[a] RM = Reichmarks. Conversion to U. S. currency was not made since this tabulation serves to indicate the relative importance of the several cost items under German conditions. Labor pay rate was 80 Pfg (0.80RM) per man hour.
Source: From data given by Hill and Campbell.[105]

and associated with mills manufacturing not less than 300 tons of sulfite pulp a day. Also, these cost figures do not include freight and sales expense and, for example, freight rates for industrial alcohol from the Pacific Northwest to the Midwest may amount to as much as about 10¢ per gallon of industrial alcohol. For comparison, Ekholm suggests that the cost of manufacturing alcohol from molasses, if this is available at 4¢ per gallon, may be calculated as 14¢ per gallon of alcohol, while the cost of producing synthetic alcohol may run between 12 and 15¢ per gallon. Ekholm concludes that under ordinary conditions, sulfite waste liquor alcohol would not have been a good competitor in the past and only time will tell what the future will bring. Tousley[204] has also reported on the economic and market aspects of sulfite waste liquor alcohol production. In considering the whole picture, it should not be overlooked that by fermentation, the biological oxygen demand of

sulfite waste liquor can be reduced to about 50%[205] which may alleviate pollution problems.

While the best processing methods available now seem quite adequate from a technical point of view, there are no new ways for major reduction in direct-process costs, except in heat savings, resulting from conduct of the fermentation process on a partially concentrated liquor which is being evaporated for burning to yield heat and perhaps process-chemical recovery. Indirectly, however, the economics of sulfite waste liquor alcohol production might be somewhat influenced by development of production and sales of other products and by-products such as yeast, carbon dioxide, and lignin derivatives.[9,123,128,143,157,172,184] A final factor to be recognized is the current trend toward construction of new wood-cellulose plants designed for the kraft, rather than the sulfite, process, although this trend may be reversed by the achievement of technical and economic success in the operation of sulfite waste liquor recovery systems.

## BIBLIOGRAPHY

1. Anon., *Chem. Inds.*, **53**, 844 (1943).
2. Anon., *Papier-Fabr.*, **12**, 947 (1914).
3. Anon, *Pulp & Paper Ind.*, **19**, No. 6, 18, 25 (1945).
4. Anon., *Pulp & Paper Ind.*, **19**, No. 10, 24 (1945).
5. Anon., *Pulp & Paper*, **22**, No. 12, 38 (1948).
6. Anon., *Pulp & Paper*, **23**, No. 9, 44 (1949).
7. Anon., *Pulp & Paper*, **24**, No. 5, 90 (1950).
8. Anon., *Pulp & Paper*, **24**, No. 13, 76 (1950).
9. Anon., *Pulp & Paper Mag. Can.*, **48**, No. 9, 70 (1947).
10. Anon., *Svensk Papperstidn.*, **45**, 337 (1942).
11. Anon., *Wochbl. Papierfabr.*, **77**, 579 (1940).
12. Abbott, W. F., *Pulp & Paper Ind.*, **20**, No. 4, 42 (1946).
13. Adler, E., *Svensk Papperstidn.*, **49**, No. 15, 339 (1946).
14. Adler, E., *Svensk Papperstidn.*, **50**, No. 11, 261; No. 11B, 9 (1947).
15. Agar, H. D., and J. L. McCarthy, *Tappi*, **34**, 126 (1951).
16. A. G. für Zellstoff und Papierfabrikation, German Patent 565,305 (1929).
17. Aktiebolaget Ethyl, Austrian Patent 27,613 (1917).
18. Aktiebolaget Ethyl, French Patents 446,717; 446,718 (1912).

19. Aktiebolaget Nordiska Armature Fabrikerna, German Patent 663,911 (1938).
20. Baum, M., J. W. Bard, J. R. Salvesen, and G. J. Brabender, *Paper Trade J.*, **126**, No. 9, 130 (1948).
21. Bergek, T., *Svensk Papperstidn.*, **46**, 148 (1943).
22. Bergson, C. R., *Finnish Paper Timber J.*, **25**, 349 (1943).
23. Bergson, C. R., *Svensk Papperstidn.*, **43**, 2 (1940).
24. Bergson, C. R., *Svensk Papperstidn.*, **44**, 29 (1941).
25. Bergström, H. O. V., *Papier-Fabr.*, **8**, 506 (1910).
26. Bergström, H. O. V., *Svensk Papperstidn.*, **24**, 927 (1921).
27. Bickell, L. K., and G. D. Tretheway, Paper presented at Can. Pulp Paper Assoc. Meeting, Montreal, Canada, February, 1951.
28. Bjørnstad, P. L., *Papir-J.*, **29**, 211 (1941).
29. Boinot, F., (to Usines de Melle), U. S. Patent 2,054,735 (1936).
30. Boinot, F., (to Usines de Melle), U. S. Patent 2,063,223 (1936).
31. Brauns, F. E., *Progress in the Chemistry of Organic Natural Products,* **5**, 175 (1948).
32. Brunes, B., *Svensk Papperstidn.*, **54**, 96 (1951).
33. Bryde, Oyvind, *Finnish Paper Timber J.*, **29**, 296, 317 (1947).
34. Buevskoi, A. V., *Bumazh. Prom.*, **16**, No. 12, 25 (1938).
35. Buevskoi, A. V., *Bumazh. Prom.*, **17**, No. 2, 31 (1939).
36. Butler, W. T., *Pulp & Paper Mag. Can.*, **50**, No. 11, 108 (1949).
37. Callaham, J. R., *Chem. & Met. Eng.*, **50**, No. 12, 104 (1943).
38. Central Laboratory of the Cellulose Industry, *Svensk Papperstidn.*, **47**, 512, 522 (1944).
39. Central Laboratory of the Cellulose Industry, *Svensk Papperstidn.*, **48**, 134 (1945).
40. Clementson, N. E., *Pulp & Paper Mag. Can.*, **19**, 316 (1921).
41. Dahl, J., *Pulp & Paper Ind.*, **20**, No. 2, 65 (1946).
42. Daniels, H. D., and J. L. McCarthy, *Paper Trade J.*, **126**, No. 5, 43 (1948).
43. Davis, M. W., *Pulp & Paper Mag. Can.*, **44**, No. 9, 656 (1943).
44. Deutsche Gold- und Silber-Scheideanstalt vorm. Roessler, British Patent 399,281 (1933).
45. Dietrich, K. R., *Svensk Papperstidn.*, **38**, 730 (1935).
46. Edling, G., *Pulp & Paper Ind.*, **19**, No. 12, 58 (1945); *Tappi Papers,* **29**, 615 (1946).
47. Ekelund, S. F. A., U. S. Patent 2,525,214 (1950).
48. Ekholm, E., *Pulp & Paper,* **25**, No. 8, 54 (1951).
49. Ekström, P. G., British Patent 6,741 (1910).
50. Ekström, P. G., Swedish Patent 30,501 (1908).
51. Ekström, P. G., Swedish Patent 31,028 (1908).
52. Ekström. P. G., Swedish Patent 33,876 (1910).

53. Ekström, P. G., Swedish Patent 51,682 (1922).
54. Ekström, P. G., Swedish Patent 74,385 (1932).
55. Ekström, P. G., U. S. Patent 1,042,332 (1912).
56. Ekström, P. G., U. S. Patent 1,050,723 (1913).
57. Ekström, P. G., U. S. Patent 1,095,830 (1914).
58. Enebo, L., E. Johnson, and H. Lundin, *Svensk Papperstidn.*, **50**, No. 11B, 72 (1947).
59. Enstrom, A. F., E. Velander, and E. Bredberg, *Svensk Papperstidn.*, **42**, 438 (1939).
60. Erdtman, H., *Tappi*, **32**, 71 (1949).
61. Erdtman, H., *Tappi*, **32**, 75, 346 (1949).
62. Ericsson, E. O., *Chem. Eng. Progress*, **43**, 165 (1947).
63. Ericsson, E. O., *Tappi Papers*, **29**, 619 (1946).
64. Feniksova, R., and R. Segal, *Spirto Vodochnaya Prom.*, **14**, No. 10, 47 (1937).
65. Flood, A. E., E. L. Hirst, and J. K. N. Jones, *Nature*, **160**, 86 (1947).
66. Foster, A. B., U. S. Patent 1,320,043 (1919).
67. Foth, G., *Handbuch der Spiritus Fabrikation*, Berlin, Paul Parey, 1929.
68. Foth, G., *Z. Spiritusind.*, **41**, 281 (1920).
69. Freudenberg, K., *Ann. Rev. Biochem.*, **8**, 88 (1939).
70. Freudenberg, K., and Th. Ploetz, *Cellulosechemie*, **19**, 131 (1941).
71. Gadd, G. O., *Finnish Paper Timber J.*, **28**, No. 7A, 61 (1946); *B. I. P. C.*, **20**, 46.
72. Gade, M., U. S. Patent 1,827,080 (1931).
73. Griffin, M. L., *Paper*, **33**, No. 9, 8 (1923).
74. Groth, B., A. Eriksson, and T. Holt, *Svensk Papperstidn.*, **47**, 405 (1944).
75. Hägglund, E., *Biochem. Z.*, **103**, 299 (1920).
76. Hägglund, E., *Die Sulfitablauge und ihr Verabeiten auf Alkohol*, 2nd ed., Braunschweig, Vieweg, 1921.
77. Hägglund, E., *Holz Roh- u. Werkstoff*, **2**, 18 (1939).
78. Hägglund, E., *Holzchemie*, 2nd ed., Leipzig, Akademische Verlagsgesellschaft m.B.H., 1939.
79. Hägglund, E., *Paper Trade J.*, **80**, No. 26, 43 (1925).
80. Hägglund, E., *Pulp & Paper Mag. Can.*, **15**, 1125, 1157, 1185 (1917).
81. Hägglund, E., *Svensk Papperstidn.*, **42**, 488 (1939).
82. Hägglund, E., *Svensk Papperstidn.*, **47**, 226 (1944).
83. Hägglund, E., *Tappi*, **33**, 520 (1950).

84. Hägglund, E., and A. M. Augustson, *Biochem. Z.*, **155**, 334 (1925).
85. Hägglund, E., and A. M. Augustson, *Biochem. Z.*, **166**, 234 (1925).
86. Hägglund, E., and A. M. Augustson, *Biochem. Z.*, **170**, 102 (1926).
87. Hägglund, E., H. Heiwinkel, and T. Bergek, *Cellulosechemie*, **21**, 108 (1943).
88. Hägglund, E., H. Heiwinkel, and T. Bergek, *J. prakt. Chem.*, **162**, 2 (1943).
89. Hägglund, E., T. Johnson, and H. Urban, *Ber.*, **63**, 1387 (1930).
90. Hägglund, E., F. W. Klingstedt, T. Rosenquist, and H. Urban, *Z. physiol. Chem.*, **177**, 248 (1928).
91. Hägglund, E., and A. Ringbom, *Biochem. Z.*, **187**, 111 (1927).
92. Hägglund, E., and T. Rosenquist, *Biochem. Z.*, **175**, 293 (1926).
93. Hägglund, E., and T. Rosenquist, *Biochem. Z.*, **180**, 61 (1927).
94. Hägglund, E., A. Soderblom, and B. Troberg, *Biochem. Z.*, **169**, 200 (1926).
95. Hägglund, E., L. Stockman, and P. Löfström, *Svensk Papperstidn.*, **53**, 551 (1950).
96. Hähnel, S., *Svensk Papperstidn.*, **47**, 75 (1944).
97. Hanson, F. S., *Paper Trade J.*, **115**, No. 24, 37 (1942).
98. Hatch, R. S., *Pulp & Paper Ind.*, **18**, No. 1, 11 (1944).
99. Hatch, R. S., *Pulp & Paper Mag. Can.*, **47**, No. 9, 80 (1946).
100. Hatch, R. S., *Tappi Papers*, **29**, 485 (1946).
101. Hawthorne, J. R., *Nature*, **160**, 714 (1947).
102. Heiwinkel, H., *Svensk Papperstidn.*, **47**, 265 (1944).
103. Hellström, N., *Svensk Papperstidn.*, **47**, 73 (1944).
104. Hibbert, H., *Ann. Rev. Biochem.*, **11**, 183 (1942).
105. Hill, A. C., and W. B. Campbell, *Final Report 51*, Item 22, British Int. Obj. Sub. Comm., London, HM Stationery Office, 1946.
106. Höpner, T., *Cellulosechemie*, **22**, 33 (1944).
107. Höpner, T., *Cellulosechemie*, **22**, 72 (1944).
108. I. G. Farbenindustrie, British Patent 496,428 (1938).
109. Jahn, E. C., *Paper Trade J.*, **120**, No. 17, 42 (1945).
110. Jensen, W., *Paper & Timber (Finland)*, **32**, 329 (1950); *B.I.P.C.*, **21**, 353 (1950).
111. Johansson, D., *Svensk Papperstidn.*, **42**, 582 (1939).
112. Johnson, M. C., and L. E. Harris, *J. Am. Chem. Soc.*, **70**, 2961 (1948).

113. Joseph, H. G., *Sewage Works J.*, **19**, No. 1, 60 (1947).
114. Kilp, W., *Wochbl. Papierfabr.*, **77**, 9 (1949).
115. Kermreuther, H., *Papier-Fabr.*, **27**, *Fest-u. Auslands-Heft*, 102 (1929).
116. Klauditz, W., *Holz Roh- u. Werkstoff*, **4**, 314 (1941).
117. Komppa, G., and Y. Tolvitie, *Ann. Acad. Sci. Fennicae*, **33A**, No. 11, 1 (1931).
118. Krohn, V., *Ann. Acad. Sci. Fennicae*, **23A**, No. 8, 3 (1926).
119. Lang, P. W., *Svensk Papperstidn.*, **50**, No. 11B, 130 (1947).
120. Larinkari, J., *Finnish Paper Timber J.*, **30**, 235 (1948); *B.I.P.C.*, **19**, 75 (1948).
121. Larsson, E. J., and J. H. Jonsson, U. S. Patent 2,496,550 (1950).
122. Lassberg, J. von, *Papier-Fabr.*, **40**, 193, 208 (1942).
123. Lassenius, T., *Finnish Paper Timber J.*, **26**, 73, 85 (1944).
124. Lassenius, T., *Finnish Paper Timber J.*, **31**, No. 7A, 4, Special Issue (April 1949).
125. Ledingham, G. A., *Pulp & Paper Mag. Can.*, **50**, No. 7, 93 (1949).
126. Leonard, R. H., W. H. Peterson, and M. J. Johnson, *Ind. Eng. Chem.*, **40**, 56 (1948).
127. Lepper, H. A., *Methods of Analysis of A. O. A. C.*, 6th ed., p. 578, Washington, D. C., Assoc. of Official Agricultural Chemists, 1945.
128. Lewis, H. F., *Paper Trade J.*, **127**, *Tappi*, 308 (1948).
129. Lochhead, A. G., and F. E. Chase, *Can. J. Research*, **23F**, 161 (1945).
130. Locke, E. G., *Pulp & Paper Ind.*, **20**, No. 1, 20 (1946).
131. Lockman, A., U. S. Patent 1,269,287 (1918).
132. Løschbrandt, F., and S. Troye, *Papir-J.*, **29**, 129, 144 (1941).
133. McCarthy, J. L., U. S. Patent 2,430,355 (1947).
134. McGovern, J. N., FIAT Final Report 487, Office of Military Govt. for Germany, Field Information Agencies Technical, Washington, D. C., 1945.
135. McGovern, J. N., and G. K. Dickerman, *Pulp & Paper Mag. Can.*, **47**, No. 6, 80 (1946).
136. McGovern, J. N., and E. L. Keller, *Pulp & Paper Mag. Can.*, **49**, No. 9, 93 (1948).
137. McGregor, G. H., *Pulp and Paper Manufacture*, Vol. 1, New York, McGraw-Hill, 1950.
138. McKee, R. H., *Paper Trade J.*, **77**, No. 15, 49 (1923).
139. Marchand, C., *Paper Trade J.*, **62**, No. 5, 201 (1916).

140. Marchand, C., U. S. Patent 1,155,256 (1915).
141. Marcus, M., *Svensk Papperstidn.*, **44**, 409 (1941).
142. Markham, A. E., Q. P. Peniston, and J. L. McCarthy, *J. Am. Chem. Soc.*, **71**, 3599 (1949).
143. Marshall, H. B., *Pulp & Paper Mag. Can.*, **50**, No. 7, 123 (1949).
144. Menzinsky, G., *Svensk Papperstidn.*, **45**, 421 (1942).
145. Mulvany, P. K., H. D. Agar, Q. P. Peniston, and J. L. McCarthy, *J. Am. Chem. Soc.*, **73**, 1255 (1951).
146. Nevrell, T. H., *Cellulosa och Papper, SPCI* **1908-1948**, 374 (1948).
147. Nilsson, N. R., Swedish Patent 84,159 (1935).
148. Noll, A., *Papier-Fabr.*, **22**, 301 (1924).
149. Oeman, E., *Svensk Papperstidn.*, **26**, 238 (1923).
150. Oliver, S. E., *Pulp & Paper Mag. Can.*, **19**, 962 (1921).
151. Olman, E., *Papier-Fabr.*, **15**, 605 (1918).
152. Opfermann, E., *Papier-Fabr.*, **31**, 533 (1933).
153. Othmer, D. F., *Ind. Eng. Chem.*, **28**, 1435 (1936).
154. Othmer, D. F., and R. L. Ratcliffe, *Ind. Eng. Chem.*, **35**, 798 (1943).
155. Palmrose, G. V., U. S. Patent 2,192,239 (1940).
156. Partansky, A. M., and H. K. Benson, *Paper Trade J.*, **102**, No. 7, 2 (1936).
157. Pearl, I. A., *Chem. Eng. News*, **26**, 2950 (1948).
158. Peniston, Q. P., and J. L. McCarthy, *J. Am. Chem. Soc.*, **70**, 1324 (1948).
159. Percival, E. G. V., *Ann Repts. on Progress Chem.*, **39**, 142 (1942).
160. Petrov, N. M., Russian Patent 52,978 (1938).
161. Ploetz, T., *Cellulosechemie.*, **21**, 14 (1943).
162. Pullianen, E., *Finnish Paper Timber J.*, **29**, 225 (1947).
163. Rahn, O., and J. E. Conn, *Ind. Eng. Chem.*, **36**, 185 (1944).
164. Richter, J., *Finnish Paper Timber J.*, **30**, 257, 269, 275 (1948).
165. Roemer, A., U. S. Patent 1,510,146 (1924).
166. Roschier, H., *Svensk Papperstidn.*, **46**, 465 (1943).
167. Roschier, H., and E. Aaltio, *Paper & Timber (Finland)*, **32**, 189 (1950); *B.I.P.C.*, **21**, 119 (1950).
168. Roschier, H., and L. Karppinen, *Finnish Paper Timber J.*, **25**, 40 (1943).
169. Rosenblad, C., *Pulp & Paper Mag. Can.*, **51**, No. 6, 85 (1950).
170. Rosten, M. M., *Chemurgic Digest*, **3**, 144, 162 (1944); *B.I.P.C.*, **14**, 428.
171. Rosten, M. M., *Pulp & Paper Mag. Can.*, **42**, 558 (1941).

172. Salvesen, J. R., *Pulp & Paper*, **24**, No. 6, 68 (1950).
173. Samuelson, O., *Svensk Papperstidn.*, **52**, 283 (1949).
174. Sandberg, E. S., N. A. E. Nilsson and J. V. Blomquist, Swedish Patent 53,317 (1923).
175. Sankey, C. A., *Can. Chem. Process Inds.*, **28**, 464 (1944).
176. Sankey, C. A., and M. M. Rosten, *Pulp & Paper Mag. Can.*, **45**, 171 (1944).
177. Sawyer, F. G., W. F. Holzer, and L. D. McGlothlin, *Ind. Eng. Chem.*, **42**, 756 (1950).
178. Schaefer, A., U. S. Patent 1,342,721 (1920).
179. Schlumberger, E., *Papier-Fabr.*, **25**, 180 (1927).
180. Schmid, W., *Wochbl. Papierfabr.*, **63**, 107 (1932).
181. Scholander, A., *Svensk Papperstidn.*, **53**, 95 (1950).
182. Schwabe, C. G., *Paper Trade J.*, **51**, No. 5, 36 (1910).
183. Schwabe, K., and E. Hahn, *Papierfabr. Wochbl. Papierfabr.*, **1944**, 351.
184. Scripture, E. W., *Paper Trade J.*, **129**, No. 17, 33 (1949).
185. Sherrard, E. C., and G. W., Blanco, *Paper*, **24**, No. 17, 15 (1919).
186. Sherrard, E. C., and G. W. Blanco, *Chem. Age (N. Y.)*, **29**, 76 (1921).
187. Sherrard, E. C., and F. W. Kressman, *Ind. Eng. Chem.*, **37**, 8 (1945).
188. Sieber, R., *Wochbl. Papierfabr.*, **51**, 2457 (1920).
189. Sirakoff, G., *Holz Roh- u. Werkstoff*, **4**, 205 (1941).
190. Smart, B., Swedish Patent 31,956 (1909).
191. Sundblad, cited by F. S. Hansen.[97]
192. Sundman, J., *Finnish Paper Timber J.*, **29**, 52 (1947).
193. Sundman, J., *Finnish Paper Timber J.*, **29**, 113 (1947).
194. Sundman, J., *Finnish Paper Timber J.*, **31**, 187 (1949); *B.I.P.C.*, **20**, 121.
195. Sundman, J., *Finnish Paper Timber J.*, **31**, 309 (1949).
196. Sundman, J., *Paper & Timber (Finland)*, **32**, 267 (1950); *B.I.P.C.*, **21**, 194.
197. Sundman, J., *Paper & Timber (Finland)*, **32**, 306 (1950); *B.I.P.C.*, **21**, 272.
198. Sundman, J., and V. Sundman, *Paper & Timber (Finland)*, **32**, 379; *B.I.P.C.*, **21**, 426.
199. Tartar, H. V., *Ind. Eng. Chem.*, **8**, 226 (1916).
200. Tomlinson, G. H., *Chem. & Ind.*, **67**, 371 (1948); *Pulp & Paper Mag. Can.*, **49**, No. 7, 63 (1948).
201. Tomlinson, G. H., *Pulp & Paper Mag. Can.*, **45**, 817 (1945).

202. Tomlinson, G. H., Canadian Patent 450,163 (1948); U. S. Patent 2,429,143 (1947).
203. Tomlinson, G. H., and L. S. Wilcoxson, *Paper Trade J.*, **110**, No. 15, 31 (1940).
204. Tousley, R. D., *Chem. & Met. Eng.*, **52**, No. 10, 120 (1945); *Tappi Papers,* **29**, 625 (1946).
205. Tyler, R. G., *Sewage Works J.*, **19**, No. 1, 70 (1947).
206. Tyler, R. G., and S. Gunter, *Sewage Works J.*, **20**, 709 (1948).
207. Uddeholms, A. B., Swedish Patent 59,818 (1925).
208. Ulfsparre, S., *The Svedberg* (Memorial Volume, 1884-1944), pp. 379-99, Uppsala, Almquist & Wiksell, 1944.
209. Vincent, R., *Paper Trade J.*, **122**, No. 9, 136 (1946).
210. Waddell, R. D., *Pulp & Paper,* **25**, No. 8, 56 (1951).
211. Waenerlund, L., *Svensk Papperstidn.*, **49**, 200 (1946).
212. Walker, R. D., and R. A. Morgan, *Paper Trade J.*, **123**, No. 6, 43 (1946).
213. Wallin, J. H., Swedish Patent 26,780 (1907).
214. Weizmann, C., U. S. Patent 2,415,777 (1947).
215. Wiley, A. J., U. S. Patent 2,429,589 (1947).
216. Wise, L. E., *Wood Chemistry,* New York, Reinhold, 1944.
217. Wurz, O., *Papierfabr. Wochbl. Papierfabr.*, **1944**, 230.
218. Wurz, O., and N. Worscha, *Papierfabr. Wochbl. Papierfabr.*, **1944**, 373.
219. Yorston, F. H., *Pulp & Paper. Mag. Can.*, **48**, No. 13, 74 (1947).
220. Zak, H., *Papierfabr. Wochbl. Papierfabr.*, **1944**, 194.
221. Zellstoff-Fabrik Waldhof and M. Gade, British Patent 341,358 (1929).

CHAPTER 5

# THE PRODUCTION OF ALCOHOL FROM WOOD WASTE

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The carbohydrates represented by sugars, starch, and cellulose are the world's most plentiful organic raw materials. Of these three, cellulose is by far the most abundant, but is the least useful as food for higher animals or for industrial fermentations. Work on the production of wood sugar has been directed toward using a waste material for these purposes.

Efforts to produce sugar and alcohol from cellulosic materials date back to the beginning of organic chemistry. The historical and technological development of this subject has been traced by many authors and is readily available.[24,36,52,57,65,101,108,120] For this reason, we shall emphasize information of technical significance and the historical aspects of the subject will not be covered in detail.

## WOOD WASTE IN THE UNITED STATES

The most comprehensive study on wood waste in the United States has been made by Winters, Chidester, and Hall[119] of the United States Forest Service. According to these workers, waste is defined as wood material from the forest that does not appear finally in marketable products other than fuel. It does not include by-

products like lath or wood flour, nor fuel wood cut for this purpose. The economics of wood-waste utilization is complicated,[118] since the supply may be located far from the market and, in some cases, significant collection and transportation costs are involved.

In 1944, the commodity drain on the forests of the United States amounted to 188.5 million tons. Imports amounted to 2.5 million tons. Of the total, only 43% appeared as products other than fuel. The logging waste amounted to 49 million tons, only 7% of which was used for fuel, and the remainder was not even removed from the woods. Primary manufacturing waste amounted to 52.9 million tons, 63% of which was burned as fuel.

Practically, the entire amount of secondary manufacturing waste, 7 million tons, was burned. None of these figures includes the waste bark, conservatively estimated at 1.8 billion cu ft. The source and disposition of all this waste is shown graphically in Figure 28.[119]

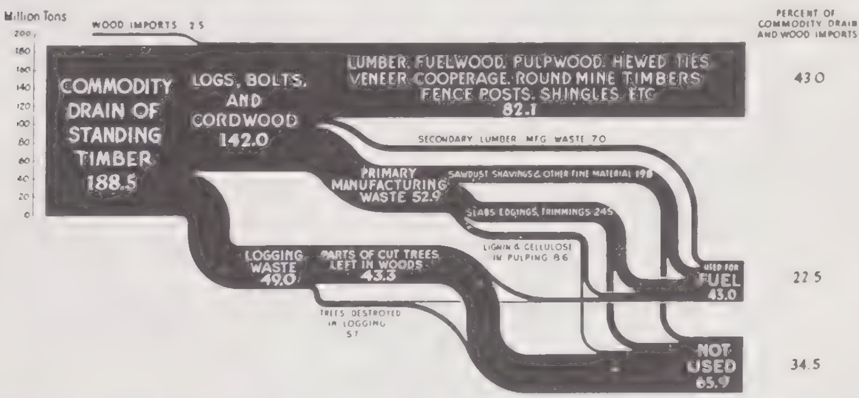


FIGURE 28. *Use and Waste in Logging and Manufacture of All Timber Products in the United States, 1944* (From Winters, Chidester, and Hall[119])

Lumber production accounts for 80% of the primary manufacturing waste. This provides the most concentrated and accessible source of material for a by-product industry. There are hundreds of locations in the United States where wood waste is available at a rate of from 50 to several hundred tons a day. This accounts for the interest in saccharification as a means of waste utilization. Winters[118] gives data on the regional distribution of wood waste produced in lumbering operations.

## THE COMPOSITION OF WOOD AND ITS SIGNIFICANCE

According to Wise,[120] the chemical ingredients of wood have been classified by Freeman as follows:

I. Main components of the cell wall
   A. Total carbohydrate fraction
      1. Cellulose
      2. Hemicellulose
         a. Pentosans
            1. Xylans
            2. Arabans
         b. Hexosans
            1. Mannans
            2. Glucosans
            3. Galactans
         c. Uronic acids
   B. Lignin
II. Extraneous materials
   A. Volatile oils and resin acids; volatile acids
   B. Fixed oils (fatty oils)
   C. Natural dyestuffs and precursors
   D. Tannins
   E. Polysaccharides and glycosides
   F. Ash (mineral salts)
   G. Organic nitrogen compounds
   H. Other organic ingredients, like resins, phytosterols, etc.

### The Total Carbohydrate

The carbohydrates of the cell wall of extractive-free wood have been named holocellulose by Ritter.[84] This consists of true cellulose, whose nature is typified by cotton, and a heterogeneous mixture called hemicellulose. Part of the hemicellulose is hydrolyzed very easily. Due to the heterogeneous nature of the total carbohydrate of wood, clean-cut separation into individual pure components is not possible. Rather, such distinctions as are made are based on arbitrary analytical methods.

Analyses by Van Beckum and Ritter,[114] shown in table 21, give the composition of certain representative woods. The holocellulose values shown include alpha-cellulose, hemicellulose, uronic acids, acetyl, and some methoxyl. The alpha-cellulose was determined by

extraction with 17.5% caustic, and the hemicellulose by difference. The holocellulose value is a measure of the sugar that can be obtained by hydrolysis.

TABLE 21. THE PERCENTAGE COMPOSITION OF CERTAIN WOODS

| | Lignin | Holo-cellulose | Alpha-cellulose | Hemi-cellulose | Pentosans | Uronic acid anhydride | Acetyl | Methoxyl in carbohydrate |
|---|---|---|---|---|---|---|---|---|
| White spruce | 26.6 | 73.3 | 49.5 | 23.8 | 10.9 | 2.68 | 2.35 | 0.70 |
| Red spruce | 26.6 | 72.9 | 48.3 | 24.6 | 11.6 | 3.20 | 2.50 | 0.92 |
| Eastern hemlock | 31.5 | 68.5 | 48.2 | 20.3 | 10.0 | 3.40 | 1.87 | 0.84 |
| Balsam fir | 30.1 | 69.9 | 44.0 | 25.9 | 10.3 | 3.08 | 2.24 | 0.41 |
| Jack pine | 27.2 | 72.5 | 49.5 | 23.0 | 12.8 | 2.92 | 1.92 | 0.75 |
| Aspen | 17.3 | 82.5 | 50.7 | 31.8 | 23.5 | 4.28 | 4.65 | 0.93 |
| Willow | 22.0 | 78.3 | | | | | | |
| Maple | 23.5 | 76.3 | 50.0 | 26.3 | | | | |
| White oak | 24.1 | 75.4 | 49.5 | 25.9 | | | | |

The hydrolysis of the polysaccharides of wood yields four hexoses: D-glucose, D-fructose, D-mannose and D-galactose; two pentoses, D-xylose and L-arabinose; and two uronic acids, D-glucuronic and D-galacturonic acids. With rare exceptions, these are the only sugars derived from any plant polysaccharide. Their configurational relationship has been shown by Norman.[74]

It is usually true that hardwoods (deciduous woods) have a lower lignin content and thus a higher carbohydrate content than softwoods (coniferous woods). The alpha-cellulose of most hardwoods and softwoods amounts to 45 to 50%. The hemicellulose content of hardwoods is somewhat higher than that of softwoods and is markedly different in composition. The two main ingredients of wood polysaccharides other than glucose are the pentoses in hardwoods and softwoods and mannose in softwoods. Analytical data on these constituents are available.[17,36,83,100,101,120,122]

### SUGARS IN WOOD HYDROLYZATES

The sugars present in the total hydrolyzate of wood have been determined by a number of workers. Hägglund[36] studied the sugars produced in the Bergius process in which nearly quantitative yields (65 to 70% reducing sugar) are obtained by the countercurrent

extraction of wood with fuming hydrochloric acid. The sugars were obtained from a mixture of spruce and pine. Similar work done at the Institute of Paper Chemistry on jack pine and birch was reported by Lewis and Davis.[110,120] They used strong sulfuric acid. Jack pine, in these studies, gave a yield of 68.7% reducing sugar and birch, a yield of 70.8%. The composition of the hydrolyzates calculated from the total sugar is given in Table 22. The determinations of glucose, galactose, xylose, and arabinose were made by fermentation techniques and were subject to limitations of the method.[120]

TABLE 22. COMPOSITION OF THE TOTAL HYDROLYZATE OF WOOD

| | Birch % | Jack pine % | Spruce and pine % |
|---|---|---|---|
| Glucose | 67.7 | 67.6 | 61.9 |
| Mannose | 1.8 | 14.1 | 24.7 |
| Galactose | 0.0 | 6.2 | 4.0 |
| Fructose | —— | —— | 1.4 |
| Xylose | 30.1 | 8.9 | 8.0 |
| Arabinose | 0.4 | 3.2 | —— |
| Total | 100.0 | 100.0 | 100.0 |

Sources: Hägglund,[36] Sutermeister,[110] Wise.[120]

The sugar obtained by the partial hydrolysis of wood were studied by Sherrard and Blanco,[107] and by Faucounau.[23] The data are given in Table 23. In these cases, conditions of hydrolysis were so mild that most of the sugar was produced from the hemicellulose. This accounts for the relatively small amount of glucose. The sugars present in sulfite liquor are derived primarily from the hemicellulose. Their composition has been determined by Hägglund and coworkers.[37]

TABLE 23. THE COMPOSITION OF SUGARS OBTAINED BY PARTIAL HYDROLYSIS OF WOOD

| | White spruce % | Bordeaux pine % |
|---|---|---|
| Glucose | 29.3 | 53.71 |
| Mannose | 37.7 | 23.76 |
| Galactose | 6.4 | 2.86 |
| Xylose | 13.3 | 12.29 |
| Arabinose | 5.4 | 7.72 |
| Volatile reducing substance | 7.9 | —— |
| Total | 100.0 | 100.34 |

Sources: Faucounau,[23] Sherrard, and Blanco.[107]

THE POTENTIAL SUGAR CONTENT OF WOOD

Wood and other lignocelluloses can be evaluated as sources of sugar and alcohol by quantitative saccharification, followed by a determination of the sugar fermentable by *Saccharomyces cerevisiae.*[69,87,89] Several woods have been analyzed in this way with the results shown in Table 24. The various woods show significant differences in yield of sugar. Hardwoods tend to give higher yields of sugar because of their lower lignin content. Aspen is noteworthy in this respect for its high potential sugar content of 75%. Large variations occur within a species. For example, Douglas-fir samples have been found to vary between 61.7 and 71.4% potential sugar.[87]

TABLE 24. YIELD OF POTENTIAL REDUCING SUGARS AND FERMENTABLE SUGARS FROM SAMPLES OF REPRESENTATIVE HARDWOODS AND SOFTWOODS

| Species | Potential reducing sugars % | Fermentability % | Potential fermentable sugars % |
|---|---|---|---|
| *Hardwoods* | | | |
| American beech | 70.1 | 75.1 | 52.6 |
| Aspen | 75.1 | 76.3 | 57.3 |
| Birch | 69.9 | 67.8 | 47.4 |
| Maple | 68.2 | 71.0 | 48.4 |
| Red oak | 63.6 | 63.0 | 40.2 |
| Sweetgum | 66.4 | 73.8 | 49.0 |
| Yellow poplar | 70.9 | 76.1 | 54.0 |
| *Softwoods* | | | |
| Douglas fir | 66.6 | 86.2 | 57.4 |
| Eastern white pine | 66.5 | 86.3 | 57.4 |
| Hemlock | 66.1 | 88.2 | 58.3 |
| Ponderosa pine | 68.0 | 82.2 | 55.9 |
| Redwood | 52.4 | 77.1 | 40.4 |
| Sitka spruce | 70.1 | 85.3 | 59.8 |
| Southern yellow pine | 64.8 | 82.0 | 53.2 |
| Sugar pine | 64.3 | 82.4 | 53.0 |

## Extraneous Materials

The extraneous components of wood, except for the ash, are soluble in neutral solvents. The water-soluble fraction of wood contains carbohydrates and polysaccharides that contribute to the

yield of sugars in wood hydrolyzates. These include starch,[11,12,34,113,120] sugars, the galactans of Western larch,[102,105,115,121] and glycosides.[120] Except in rare cases, Western larch, for example, the amount of sugar contributed is too small to be of significance.

The nitrogen content of the more common woods, growing in the temperate zone, is low, falling between 0.1 and 0.3%.[101] This includes protein and nonprotein ingredients.

There is a wide range in the ash content of various woods. Data on the mineral constituents of wood have been compiled by Schorger,[101] Hägglund,[36] and Wise.[120] The ash content of most North American species is below 1%. Many elements have been reported, but the main ingredients include calcium oxide, often amounting to half of the total ash, and potassium oxide, which usually amounts to 10 to 30% of the ash. Phosphorus pentoxide shows a large variation, but is frequently present to the extent of 5 to 15% of the ash.

Oils, resins, terpenes, tannins, and other extraneous components of wood listed in the classification given previously are probably of significance in determining potential applications of sugar from various woods. An extensive review of these substances has been presented by Kurth.[120]

### Lignin

Lignin is the main noncarbohydrate ingredient of wood and other plants. It is essentially a high polymeric, aromatic substance and most of it remains after all hydrolysis procedures as an insoluble residue, amounting to 20 to 30% of the wood. The chemistry of lignin has been reviewed by Phillips[120] and Freudenberg.[29]

A number of proposals have been made for the utilization of lignin, among which are its use in plastics,[71,106] as a soil conditioner,[39,90,125] in the production of charcoal,[79,80,120] and in the preparation of hydrogenation products.[5,15,42,47,48,88] The successful use of lignin could have a large effect on the economics of wood saccharification.

## THEORY OF CELLULOSE HYDROLYSIS

The resistance of cellulose to hydrolysis is the most important factor in determining the cost, the methods of production, and the

character of the wood sugar. Detailed studies on the kinetics of cellulose hydrolysis[28,30,32,33,63,64,85,123,124] and of starch hydrolysis [31,32,56,70] have been made. An understanding of problems of wood hydrolysis can be obtained by comparing it with the familiar behavior of starch.

Starch is hydrolyzed commercially at pH 1.8 to 2.0 under 30 psi steam pressure for 20 to 30 minutes.[56] In order to hydrolyze true cellulose at a similar rate, it is necessary to employ five times higher steam pressure and a hydrogen-ion concentration roughly ten times as great as that needed for starch hydrolysis. Extrapolation to similar conditions indicates a difference in ease of hydrolysis of hundreds or thousands of times.

Under ordinary conditions of dilute-acid batchwise hydrolysis, the yield of sugars from cellulose is only a little more than one-third of that obtainable from starch. This is not due to the slower rate of sugar production, but rather to the lower ratio of sugar production to destruction, which places a theoretical limitation on the maximum yield obtainable.

Haworth[50] has suggested that the differences between starch and cellulose can be accounted for by their configuration. The $\beta$-glycosidic linkages of cellulose allow the molecules to be stretched into extended chains, but in starch only a zigzag arrangement is possible.[4,50] This results in more hydrogen bonding in cellulose than in starch, and, thus, in lower solubility, less tendency to swell, and less accessibility to the reagent.

## PROCESSES FOR THE HYDROLYSIS OF WOOD

All processes for the hydrolysis of wood fall into the classes of strong-acid or dilute-acid methods. In strong-acid methods, a large amount of reagent is required, but the yields are high and the product is comparatively free of degradation products. In dilute-acid methods, much less reagent is required, but yields are lower and the product is contaminated with undesirable by-products.

### Strong-Acid Processes

Only one strong-acid wood-saccharification process, the Bergius process, has become industrially important. Many others have been investigated, but are not used commercially. A strong-acid process

for the saccharification of agricultural wastes has been described recently.[18]

THE BERGIUS PROCESS

The Bergius process makes use of fuming hydrochloric acid, which dissolves cellulose readily. The process is based on the early work and patent of Willstätter.[116,117] Experimental work was carried out in Germany and in Switzerland.[38,73] A small demonstration plant was built at Rheinau in 1933 and in 1937, the process was described by Berguis.[7] In 1940 a full-scale plant was built at Regensburg, Germany. This plant used the same process as was used at Rheinau and apparently there has been no important advance since that time. Information about the operation of the Regensburg plant was obtained following the end of World War II.[90]

The Regensburg plant has twenty-eight 50-cu m extractors in two parallel batteries, arranged for countercurrent extraction. Half of the extractors are in the process of saccharification, while the other half is undergoing washing to remove the acid from the residue. Periodically, an extractor of washed lignin residue is discharged, another extractor is taken from the saccharification and put in the washing process, and a third extractor of fresh wood is put into the saccharification process. The total cycle for each extractor is 55 hours. The fuming hydrochloric acid, after passage through the series of extractors, contains 32% of carbohydrate by volume. This solution is distilled under vacuum at 36°C. Most of the acid is recovered in this way and additional acid is recovered in a spray drier.

The spray-dried hydrolyzate contains glucose, mannose, xylose, galactose, and fructose, mainly in tetrameric form. One or 2% hydrochloric acid remains. This hydrolyzate can be converted easily and without loss into the monomers by dilution with 3 volumes of water and heating at 120°C for ½ hour.[7] The crude product, consisting of tetrameric sugars, can be neutralized and used as fodder without further hydrolysis. The sugar from the secondary hydrolysis can be used in a number of fermentation processes.

At the Regensburg plant, all of the output was in the form of fodder yeast, although successful alcohol production has been demonstrated. The plant is reported to have cost 20,000,000 marks, or about 8,000,000 dollars.

The yeast, amounting to 500 metric tons a month, was produced

from 1,000 tons of reducing sugar.[13,90] The raw material requirements were as follows:

| | |
|---|---|
| Wood | 6,500 Raum meters (1,800 cords) |
| Illuminating gas | 100,000 cubic meters |
| Chlorine | 300 metric tons |
| Lime | 100 to 200 metric tons |
| Hard coal | 2,400 metric tons |
| Ammonia liquid | 75 metric tons |
| Ammonium sulfate | 10 metric tons |
| Diammonium phosphate | 30 metric tons |
| Trisodium phosphate | 0.5 metric ton |
| Magnesium sulfate | 8 metric tons |
| Potassium chloride | 15 metric tons |
| Antifoam | 17 metric tons |

The wood was obtained as solid wood rather than as sawdust or another form of waste. This was necessary for proper control of the particle size in the diffuser and greatly increased the raw-material cost.

## Dilute-Acid Processes

Dilute-acid processes have been used to a much greater extent than strong-acid methods. In spite of lower yields, they have economic advantages, particularly in plant costs.

### The Single-Stage Batch Process

This process of wood hydrolysis was the first successful method for making sugars from wood. It is very simple and, with some improvement,[81] it may still be economically sound in locations with a large wood supply.

The process was applied first in a plant at Georgetown, South Carolina, then in one at Fullerton, Louisiana. These plants operated just prior to and following World War I and, according to Sherrard and Kressmann,[108] produced 5,000 to 7,000 proof gallons of alcohol a day. During the war, the lumber mills with which these plants were associated overcut their holdings. This, together with a decrease in the price of blackstrap molasses, resulted in closing both plants.

The Georgetown plant has been described by Foth[24] and by Demuth.[16] Experimental work was carried on concurrently with the operations and was described by Kressmann.[57] In 1921, Sherrard

presented an engineering study of the process.[104] The cost of production was then estimated at 25¢ per gallon of 190° proof alcohol. The operation of such a plant has been described by Kressmann.[57] The raw materials required for the production of 3,600 gal of alcohol per day were estimated at:

| | |
|---|---|
| Wood (dry basis, not over 10% bark) | 180 tons |
| Sulfuric acid (60° Baumé) | 3.9 tons |
| Lime | 2.2 tons |
| Molasses | 150 gal |
| Malt sprouts | 317 lb |
| Ammonium sulfate | 175 lb |

At the Georgetown plant, the wood was processed on a 1-hour cycle in four spherical digesters, each holding 4,700 lb of dry wood. The sugar was extracted in a battery of eight 150-cu ft cells, arranged for countercurrent extraction. About 96% of the sugar was extracted to give a solution containing about 12% total solids, 9% reducing sugars, and 6% fermentable sugars. This was neutralized to 4.5 acidity; that is, 20 ml required 4.5 ml of N/10 alkali. The recommended design figure in such a plant was 20 gal of alcohol per ton of wood.

The yield obtained from various woods by this process has been determined in experimental equipment by Kressmann[57] and Sherrard.[104]

### The Single-Stage Continuous Process

A wood-hydrolysis process has been described in which a slurry, consisting of 8 to 10 parts of dilute acid to 1 part of sawdust, is pumped through a heat exchanger.[54,55,71,72] This has not been used commercially. The process has the disadvantage of giving dilute sugar solutions because of the high water-to-wood ratio.

### Percolation Processes

In the batchwise hydrolysis of cellulose, the rates of sugar production and destruction are of similar magnitudes. As a result of this, the yield from batchwise hydrolysis as ordinarily practiced is only about one-third of the theoretical. The only way to improve the yield is to make the ratio of production to destruction more favorable. The basic advantage of strong-acid hydrolysis, such as the Bergius process, is the high yield resulting from a high ratio of production to destruction. Similar advantages are obtainable in

a dilute-acid percolation process developed and put into commercial use by Scholler.

The basic principle involves the hydrolysis of wood in a pressure vessel by a percolation process in which dilute acid is injected into the top of the vessel and withdrawn through a screen in the bottom. The earliest development was described by Bausch[6] and Lüers.[63,64] In this way, sugar production and extraction go on simultaneously and the sugar is withdrawn and cooled as soon as possible after it is formed. The process was originally intended to make use of a battery of percolators arranged for countercurrent extraction. These plans were later discarded in favor of percolators operated separately.

The countercurrent saccharification of wood with sulfur dioxide solutions has been carried out recently by Ant-Wuorinen.[1,2,3]

The industrial development of the Scholler process was described by Schaal,[93] Fritzweiler and Rochstroh,[26] Lüers,[65] Scholler,[94,95] and Fritzweiler and Karsch.[27] These sources do not give detailed information on the process, but in 1945, operating details of plants in Tornesch and Holzminden, Germany, were obtained.[35,90]

**European Scholler Process Plants.** There are three Scholler plants in Germany and one in Switzerland. One was reportedly built in Korea and one was under construction in Italy.[90] The methods used at the Tornesch and Holzminden plants are similar to those at the other plants. The specific details of the operation can be obtained from the before-mentioned references. Only a summary of the procedure will be given here.

A Scholler plant contains six or eight 50-cu m digesters, constructed of steel and lined with acid-resistant tile. The diameter of these digesters is 2.4 m and the over-all height about 13 m.[93] The top of the digester has steam and vent lines and a line for the injection of dilute acid. The bottom is equipped with a filter cone and with a quick-opening valve for discharging the lignin residue. The digester is loaded with 9 to 10 metric tons of sawdust, shavings, and chips to a density of 180 to 200 kg of dry wood substance per cu m (about 12.5 lb per cu ft). In order to reach this density, the percolator is filled and subjected to a sudden steam shock to compress the charge. This is repeated until the desired loading is obtained. After the percolator is filled, the wood is heated with direct steam to 134°C. A charge of dilute acid, containing 1.4% sulfuric acid, is then injected at a temperature lower than that of the percolator

contents and the injected acid is heated by steam from the bottom until the desired temperature is reached. The solution is then "pressed" from the percolator by applying steam to the top of the charge. This operation is repeated approximately nineteen times with 0.6% acid at temperatures increasing to a maximum of 184°C. The total amount of liquor obtained per percolation is about 120 metric tons (120 cu m) from 10 metric tons of wood.[35,90]

The Holzminden plant, the newest in Germany, made use of six 50-cu m percolators. The design of the plant was for an output of 360,000 l of absolute alcohol per month, using 1,800 metric tons of wood. During 1943, the year of the highest production since it began operation in 1939, the plant operated 330 days of the year, used 90% of the wood it was designed to use, and produced 76% of the design capacity of alcohol. The average time required per percolation was nearly 24 hours. During 1939, the first year of its operation, the yield was 18.1 l of alcohol per 100 kg of wood. One hundred fifty-five workers were required to operate the plant. It is unfortunate that the war complicated the operation of this plant, since the process cannot be properly evaluated with the data available.

The wood, chemical, and energy requirements for 1 month for ideal operation at the Holzminden plant[90] are as follows:

| | |
|---|---|
| Dry wood | 1,800 metric tons |
| Sulfuric acid | 120 metric tons |
| Calcium oxide | 90 metric tons |
| Calcium carbonate | 110 metric tons |
| Superphosphate | 1.5 metric tons |
| Ammonium sulfate | 2.5 metric tons |
| Antifoam | 1.5 metric tons |
| Trisodium phosphate | 1.5 metric tons |
| Hard coal | 600 metric tons |
| Steam | 8,000 metric tons |
| Water | 50 cu m per hour |
| Electricity | 160,000 kilowatt-hours |

Greaves[35] gives data indicating a higher chemical requirement in actual operation.

The plant at Tornesch was a demonstration plant and it was not operated on a scale that would provide satisfactory information on the economics of the process. Complete data on the Dessau plant are not available.

The Scholler plant at Ems, Switzerland,[90,111,112] has proved very successful. It was designed to produce 500,000 l of absolute alcohol per month, and it regularly produces 800,000 l. The process is essentially as described except that the time has been decreased and the yield has been increased. The average yield at the Ems plant is 21 l of absolute alcohol per 100 kg of wood. This corresponds to 53 gal of 190° proof alcohol per ton. This is much better than the wartime record of the German plant described, but it is essentially the yield predicted by Fritzweiler and Karsch.[27] The Ems plant has eight percolators holding 10 metric tons per charge. The monthly production given before requires that the percolation cycle is completed in an average of 15 hours.

**Recent Work on Wood-Sugar Production in the United States.** In 1935, the Cliffs Dow Chemical Company of Marquette, Michigan, obtained the rights to the Scholler process in the United States. A modified Scholler process was studied on a pilot-plant scale, but it was not used commercially.

In 1943, the War Production Board recommended a study of the Scholler process by the U. S. Forest Products Laboratory in the pilot-plant facilities at Marquette, Michigan. The Vulcan Copper and Supply Company was requested to follow the pilot-plant operations and to prepare a process report that could serve as a basis for the engineering design of a commercial plant. As a result of these investigations, the War Production Board recommended to construct and operate a commercial plant using the modified Scholler process as insurance against possible future shortages of raw materials for alcohol production. In 1944, design and construction was begun by the Vulcan Copper and Supply Company at Springfield, Oregon. The process was described by Faith[20,21] and compared with the Scholler process as it was used in Germany. The operation of this plant will be described later in this chapter.

*The Madison Wood-Sugar Process.* The pilot-plant operations, begun at Marquette, Michigan, were transferred to Madison, Wisconsin, where additional work was conducted. The details of the process devised by Harris and coworkers at the Forest Products Laboratory are readily available.[40] In brief, the process involves the use of a wood waste containing up to 25% bark as raw material. This is loaded into the 27 cu ft percolator to a density as high as 18 lb per cu ft. Dilute acid of an initial concentration of approxi-

mately 2% is introduced, which is diluted to 0.5 to 0.6% concentration as soon as the average acidity in the digester reaches that value. After a short initial holding period, continuous injection of the dilute acid and withdrawal of the product is begun during which the temperature is raised gradually from 150° to 185°C. A total of about 10 parts of water is used per part of wood. At the end of the percolation, after the charge has been drained, a large valve in the bottom of the percolator is opened to discharge the lignin into

TABLE 25. HYDROLYSIS OF WOOD BY THE MADISON WOOD-SUGAR PROCESS

| Type of wood product | Hydrolysis time hours | Yield of sugar % | Sugar concentration % |
|---|---|---|---|
| White-spruce chips | 3.1 | 54.2 | 5.1 |
| Douglas-fir chips | 3.0 | 52.5 | 5.3 |
| Douglas-fir sawdust | 3.1 | 44.7 | 4.9 |
| Douglas-fir hog fuel | 3.0 | 38.7 | 5.1 |
| Douglas-fir bark | 2.9 | 15.3 | 2.3 |
| Southern yellow-pine wood waste | 3.3 | 50.0 | 4.8 |
| Southern yellow-pine sawdust | 3.1 | 47.5 | 4.6 |
| Ponderosa-pine chips | 3.0 | 51.5 | 5.4 |
| Eastern white-pine sawmill slabs | 3.1 | 44.6 | 4.6 |
| White-fir chips | 3.0 | 53.8 | 5.4 |
| Western white-pine chips | 3.0 | 46.6 | 5.0 |
| Sugar-pine chips | 3.0 | 46.9 | 4.0 |
| Western hemlock chips | 3.0 | 51.5 | 5.0 |
| Western larch chips | 3.0 | 54.0 | 4.9 |
| Western larch sawmill slabs | 3.0 | 42.0 | 4.9 |
| Lodgepole-pine chips | 3.0 | 51.0 | 4.9 |
| Spent-turpentine chips, longleaf-pine stumps | 3.1 | 40.0 | 4.8 |
| Western red-cedar chips | 3.1 | 46.9 | 4.3 |
| Redwood chips | 3.1 | 42.6 | 4.0 |
| Mixed southern oak shavings | 3.0 | 51.0 | 5.0 |
| Mixed southern oak sawdust | 3.0 | 46.9 | 5.1 |
| Mixed southern oak sawmill waste | 3.1 | 42.9 | 4.7 |
| Sugar-maple sawmill waste | 3.0 | 48.0 | 4.8 |
| Yellow-birch sawmill waste | 3.1 | 49.5 | 5.3 |
| Beech sawmill waste | 3.1 | 46.5 | 5.0 |

Source: Reference 40.

a receiver. The total percolation in the pilot operation requires approximately 3 hours.

The sugar solution removed from the bottom of the percolator is passed to a flash tank where the pressure is reduced to 30 psi. The solution is neutralized and filtered at this pressure to avoid subsequent scaling in the stills. The high-temperature neutralization has also been found useful in improving the fermentability.[58]

Harris and Beglinger[40] give data on the potential sugar contents of various forms of wood waste and on the sugar actually produced in pilot-plant operation. The yield of sugar obtained from certain typical wastes is shown in Table 25. Douglas-fir waste, containing 25% bark, yields 0.38 gal of alcohol per cu ft of percolator capacity per percolation. The actual reaction time in these operations was 3 hours. Allowing four such percolations per day, a yield of 1.52 gal per cu ft per day could be obtained. The Holzminden plant in Germany was designed to produce 0.30 gal per cu ft of percolator capacity per day. The Springfield plant was designed on the basis of 1.07 gal per cu ft per day.

*The Work of the Tennessee Valley Authority on Wood Sugar.* Gilbert, Hobbs, and Levene[34a] of the Tennessee Valley Authority have studied a modification of the Madison wood-sugar process with primary emphasis on the production of molasses. Nontimber grades of oak waste were used as a raw material. The hydrolyzate from hardwoods generally is high in pentose and, therefore, less desirable than softwood hydrolyzates for alcohol production.

The pilot plant used in the Tennessee Valley Authority studies made use of simpler equipment and was arranged for the recycling of dilute liquors. The process modifications developed in this work are probably applicable to softwood hydrolysis.

## EXPERIMENTAL WORK ON WOOD-SUGAR FERMENTATION

The sugars present in wood polysaccharides have been discussed in the first part of this chapter. The total hydrolyzates of softwoods are from 77 to 88% fermentable by *Saccharomyces* yeasts. Similar hydrolyzates from hardwoods are from 65 to 75% fermentable.[87] The alcoholic fermentation of softwood hydrolyzates obtained by percolation methods[27,40,41] show fermentabilities of about 80% [43,46,51,58] the remainder being mainly unfermentable pentoses.

The bacterial fermentation of such hydrolyzates with *Clostridium butylicum*[109] or *Aerobacter aerogenes*,[77] or the aerobic fermentation with *Torulopsis, Monilia* or *Candida*, utilized 90 to 96% of the total reducing substances.[44,45,49,78,86]

Successful wood sugar fermentations have been carried out in the laboratory and industrially with consistent results once the necessary fermentation conditions were defined. The yields, based on sugar utilized, are similar to those obtainable from other more favorable substrates, but usually special treatment of the substrate or acclimatization of the organism is necessary for satisfactory results.

## Fermentability of Wood Hydrolyzates

The literature on the dilute-acid hydrolysis of wood emphasizes the fact that the sugar produced is difficult to ferment by conventional methods.[27,93] Leonard[58] found that yeast inocula that would have been adequate for grain worts failed to ferment untreated hydrolyzates unless a carefully controlled slow-feeding technique was used.

The first successful industrial wood-sugar production and fermentation was in the two plants operated in the United States during World War I. Sugar production at these plants was by a dilute-acid, single-stage batch hydrolysis. The fermentations were started by the addition of wood sugar to a molasses medium inoculum over a period of 24 hours. The fermentations were then continued an additional 72 hours.[16,24] Following this, German workers tried to correct the toxic character of the dilute-acid hydrolyzates.[22,26,61,66,67,93,96,97] This subject was reviewed by Leonard and Hajny,[58] who listed four potential sources of toxic substances: carbohydrate decomposition, lignin decomposition, extraneous materials present in wood, and metal ions from equipment corrosion.

Reducing sugars account for about 75% of the organic material present in wood hydrolyzates. The balance consists of organic acids, dissolved lignin, furfural, hydroxymethylfurfural, extraneous material from the wood, and various unknown decomposition products.

The main soluble decomposition product of pentoses in the presence of acid under a wide variety of conditions is furfural, and that of hexoses is hydroxymethylfurfural, which is readily converted to formic and levulinic acids. The second acid is relatively nontoxic.

The automatic neutralization of hydrolyzate at high temperature with calcium hydroxide[40] under inaccurate control may result in some local overneutralization. High pH at high temperature can cause the formation of many acids, aldehydes, and other compounds. In spite of this, the net result of high-temperature neutralization has been favorable.

Lüers *et al.*[66,67] determined the concentration of various substances required to cause a 25% inhibition in yeast propagation or in fermentation, with the results shown in Table 26. The Scholler-process hydrolyzates were found to contain furfural concentrations varying from 0.015 to 0.04 g per 100 ml and higher.

TABLE 26. TOXICITY OF VARIOUS COMPOUNDS FOR YEAST

| Substance | *Concentration Causing 25% Inhibiting Effect* In yeast propagation g per 100 ml | In yeast fermentation g per 100 ml |
|---|---|---|
| Furfural | 0.110 | 0.0740 |
| 5-Hydroxymethylfurfural | 0.140 | 0.2600 |
| Menthol | 0.011 | 0.0096 |
| Borneol | 0.033 | 0.0130 |
| *d*-Pinene | 0.002 | 0.0085 |
| Pyrogallol | not tested | 0.3700 |
| Phloroglucin | not tested | 1,3600 |
| Gallic acid | not tested | 0.2300 |
| Tannin | not tested | 0.0250 |
| Terpineol | 0.037 | not tested |
| Vanillin | 0.063 | not tested |
| Eucalyptol | 0.065 | not tested |

Source: Lüers, Fries, Huttinger, Morike, Enders, Karnbach, and Wieninger.[67]

Among the treatments or conditions found to be advantageous in reducing toxicity of hydrolyzates are: (1) steam distillation,[58] (2) acclimatization of the yeast,[43,46] (3) detoxification reactions brought about in the presence of actively fermenting yeast,[58,66,67] as illustrated by the reaction of furfural[62] and other phytochemical reductions,[14,68,82] (4) treatment with lime,[96] (5) precipitation with proteins,[96] (6) adsorption on surface-active materials,[96] (7) increasing the pH, filtering and readjusting the pH,[75] (8) use of large yeast inocula,[26,27] (9) adjustment of oxidation-reduction potential, e.g., by the addition of sulfur dioxide,[58] (10) holding at high tem-

peratures or neutralization at high temperatures,[40,58] and (11) extraction or sulfide precipitation.[8]

**Yeast Strains Adaptable to Wood Sugar**

Johnson and Harris[51] investigated the ability of twenty strains of yeast to grow and produce alcohol on Douglas-fir hydrolyzate. The hydrolyzate was neutralized and filtered at a temperature of approximately 130°C, but was given no further treatment beyond the addition of nutrient salts. Experiments on yeast growth were made with 200 ml quantities in shake flasks, the yeast being centrifuged and transferred to fresh wort daily. Several of the strains grew poorly on the first transfer, but by the twelfth to fifteenth transfer, all of them utilized from 79 to 94% of the total sugar. The cells quickly became permeated or coated with dark-colored material, but they remained active throughout the investigation. It appears that a wide variety of yeasts can be grown satisfactorily on wood sugar.

Wood hydrolyzate has also proved satisfactory for the production of fodder yeast by aeration. Yeast strains such as *Torulopsis utilis* are capable of utilizing the pentoses, as well as the hexoses, under these conditions. The pentoses in stillage from alcoholic fermentation may be utilized in this way to reduce the waste-disposal problem.

The same twenty strains of yeast were tested by Johnson and Harris for their ability to produce alcohol, using the same wort under anaerobic conditions. An initial inoculum of 1% of cell volume was used and the yeast was centrifuged and transferred daily. More than half of the cells became small and discolored, but the cultures remained active. Certain strains showed a very low conversion at first, but they developed the ability to produce alcohol efficiently after twelve transfers. All of the strains eventually utilized from 77 to 84% of the reducing sugar present and most of them gave satisfactory yields of alcohol. This indicates that many yeasts can be adapted to wood-sugar fermentation.

Harris, Hajny, Hannan, and Rogers[43] conducted batch fermentations of Douglas-fir hydrolyzate with *Saccharomyces cerevisiae* in quantities of several hundred gallons. The yeast was recovered and recycled in continuous transfer. The alcohol yield was 39.2 to 40%, based on total sugar, and 47 to 47.9%, based on the fermented sugar. The yield rose for the first few transfers and then remained

constant through the transfers. When 4% of yeast by volume was used, the fermentation was complete in 5½ hours.

In later work,[46] these investigators conducted continuous fermentations with *Torulopsis utilis.* Six 10-gal fermentors, connected in series, were inoculated with 2% yeast and operated with a

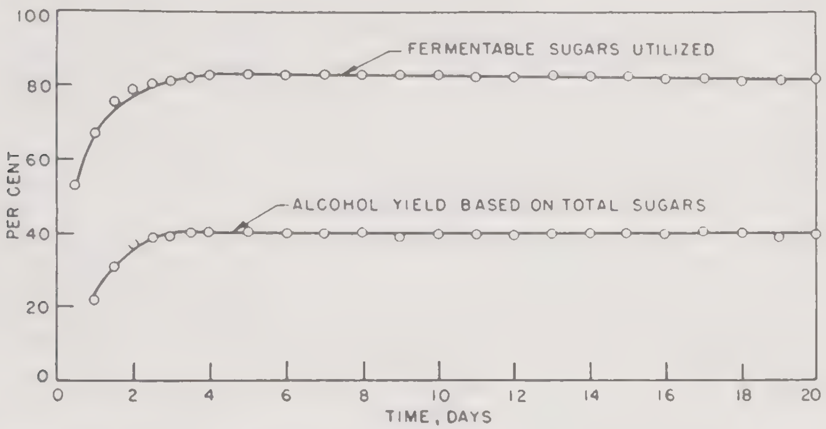


FIGURE 29. *Continuous Fermentation of Wood Hydrolyzate by* Torulopsis utilis *in six 10-gal Tanks*

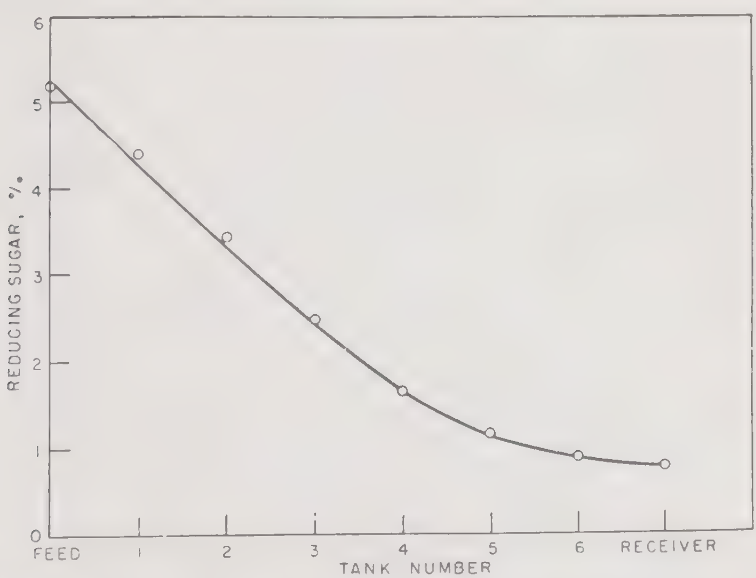


FIGURE 30. *Reducing Sugar in Each Tank in Continuous Fermentation (sixteenth day)*

throughput of 60 gal a day. After the tenth day of a 20-day run, 80 to 82% of the sugars was utilized and an alcohol yield of 40% was obtained, based on the total sugar. Figure 29 shows the sugar utilized and the yield of alcohol as the experiment progressed. Figure 30 shows the sugar concentration in each of the fermentors.

In summarizing the experimental work on the fermentation of dilute-acid wood hydrolyzate, it is apparent that this substance, because of the presence of toxic compounds and possibly because of an unfavorable oxidation-reduction potential, is inhibitory to yeast activity. This inhibition can be overcome by the use of large inocula or by other means mentioned previously.

Experience to date shows that wood hydrolyzates that are being converted to yeast or alcohol are so inhibitory to other microorganisms that continuous fermentations can be carried out for a long period of time with little trouble due to infection. This is an advantage that in large measure compensates for the other disadvantages of wood hydrolyzate.

## COMMERCIAL FERMENTATION OF WOOD HYDROLYZATES

The first industrial-scale fermentation of wood hydrolyzate was carried out at Georgetown, South Carolina, and at Fullerton, Louisiana.[16,24,36] The sugar was produced by the batchwise hydrolysis described previously. Concurrent experimental work was described by Kressmann.[57] The directions given by Kressmann for laboratory-scale fermentation illustrate the best technique available, a slow-feed fermentation requiring a total of about 4 days. The technical and economic aspects of such an operation have been described by Sherrard.[104]

### The Production of Alcohol from Wood Waste at the Springfield Plant

At the Springfield, Oregon, plant, it was expected to operate with continuous fermentation similar to that used in Europe. The plant, however, was never in full-capacity operation, nor was the wood sugar produced at a steady rate. Consequently, batchwise fermentations were carried out with recycling of the yeast. This method of operation is probably inferior to continuous operation, but observations that were made for the batch operation are applic-

able to wood-sugar fermentations in general. Because of a lack of such information in the literature, the operation will be described in some detail.

The process is illustrated in the flow sheets of Figures 31, 32 and 33 and the photographs of Figures 34 and 35. The plant had five 2,000 cu ft percolators, each of which held 12.5 tons of bark-free dry wood substance. The design throughput was 221 tons of wood a day, with a production of 10,700 gal of 190° proof alcohol and 800 gal of "heads" fraction. The incoming wood was passed through screens and the oversize material went through a hammer mill. A bin house provided storage capacity for the operations of nearly 1 day. The percolators were charged through a 16 in. motor-driven gate valve by a conveyor passing over a weightometer. The charge was compressed by steam shocking, as has been described. The raw material consisted of Douglas-fir hogged waste. Approximately one-tenth of the charge consisted of chips passing a 2-in. screen. This was put in the bottom to serve as a filter bed.

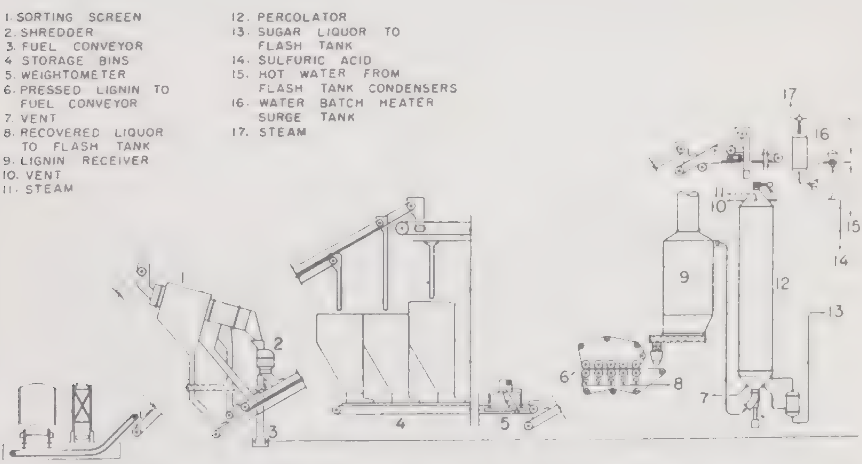


FIGURE 31. *Wood-Handling and Percolation Sections of a Wood-Hydrolysis Alcohol Plant* (Courtesy—The Vulcan Copper & Supply Co., Cincinnati, Ohio)

The percolation with 0.5 to 0.6% sulfuric acid was carried out at temperatures gradually increasing from 160° to 182°C. A level control at the bottom of the percolator regulated the flow out of the percolator. After the hydrolysis was finished, discharge of the lignin

was accomplished by means of a quick-opening pneumatic valve at the bottom of the percolator. The acid hydrolyzate was prepared for fermentation by neutralizing with calcium hydroxide under 30

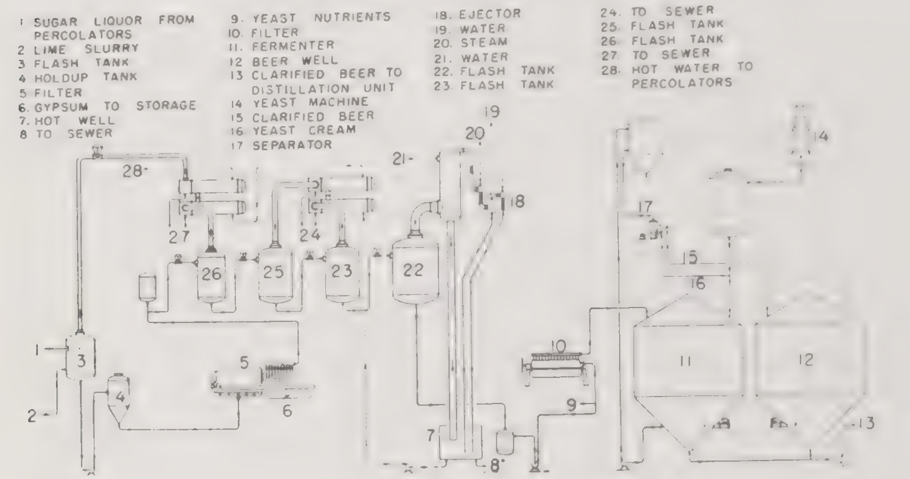


FIGURE 32. *Liquor-Preparation and Fermentation Sections of a Wood-Hydrolysis Alcohol Plant* (Courtesy—The Vulcan Copper & Supply Co., Cincinnati, Ohio)

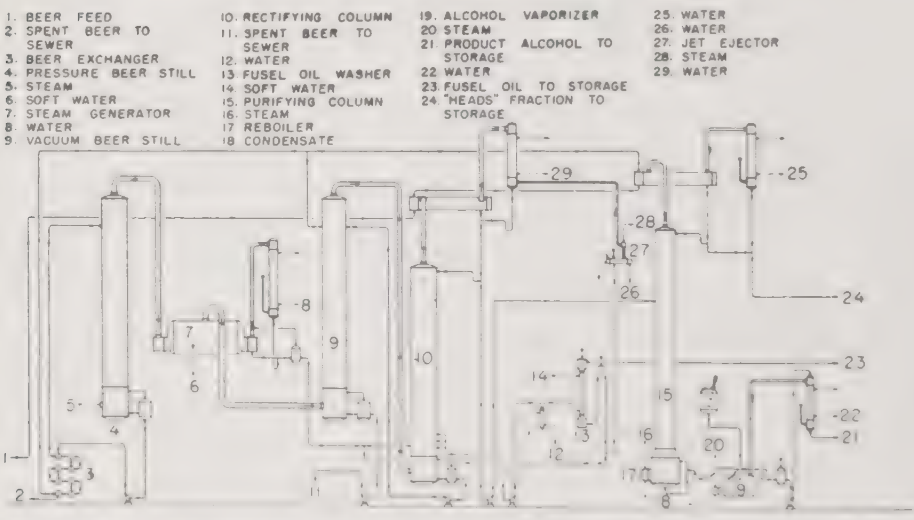


FIGURE 33. *Distillation Section, Employing the Vulcan Vapor-Reuse Process, in a Wood-Hydrolysis Alcohol Plant* (Courtesy—The Vulcan Copper & Supply Co., Cincinnati, Ohio)

to 40 psi steam pressure. Pilot-plant work had shown this to give a solution easier to ferment and to decrease the solubility of the calcium sulfate. The second feature was particularly important

FIGURE 34. *Percolation Section. View of the Percolator Charger Valve and the Back of the Percolator Panel from the East End of the Fifth Foor of a Wood-Hydrolysis Alcohol Plant* (Courtesy—The Vulcan Copper & Supply Co., Cincinnati, Ohio)

FIGURE 35. *Fermentation Section. Yeast Separators in the Yeast Room; Magne Yeast Machine on Left* (Courtesy—The Vulcan Copper & Supply Co., Cincinnati, Ohio)

in this plant, because two-stage beer stills were used, the first of which operated under pressure. The vapors from this still produced steam in a reboiler for the operation of the second beer still. This system resulted in marked steam economy, but to avoid scaling in the stills, it was desirable to remove as much calcium sulfate as possible. The neutralized liquor, still under 30 to 40 psi steam pressure, passed through leaf filters and then through a series of four flash tanks to cool it to fermentation temperature. Just prior to fermentation, the solution was filtered again to remove a fine black precipitate that separated on cooling and exposure to air. When yeast was added immediately after the second filtration, the solution became lighter colored and very little additional precipitate was formed. The prepared solution showed a titrable acidity of 2.0 ml of 1 *N* sodium hydroxide per 100 ml, and the pH was between 4.5 and 5.0.

Because of problems in plant start-up at partial capacity, there were wide variations in the quality of the sugar solutions produced. The most serious of these resulted from excessive hold-up time in the percolator and from inaccurate control in the high-temperature neutralization. In spite of large variations, there was only one instance in which the material failed to ferment properly, and this was definitely traced to excessive acidity.

Regarding the nature of the sugar subjected to fermentation, it was concluded that fermentations proceeded about as predicted from pilot-plant work.[40,58] Variable control in sugar production resulted in varying rates of fermentation, but large variations in quality of the substrate could be tolerated.

The yeast used at the Springfield plant was a Java molasses strain of *Saccharomyces cerevisiae.* This strain, in limited laboratory tests with plant hydrolyzates at Springfield, gave results somewhat superior to those obtained with strains of *Torulopsis* or other strains of *S. cerevisiae* employed previously in pilot-plant work.[43,46] The inoculum was grown in the laboratory in two stages of anaerobic and one stage of aerobic fermentation. The culture was then transferred to a Magne propagator of 494 gal capacity in the plant. The culture from the Magne propagator was divided between two 6,500-gal propagators equipped with carbon air spargers. The air supply was sterilized with cresol solution in a spray tower. No contamination was encountered in the yeast-inoculum production. Sufficient inoculum was grown on molasses to provide 1% of yeast

by volume in the fermenting wood sugar. Yeast was subsequently recovered from the fermented beer and recycled.

In wood sugar fermentation, a yeast-reuse or recycling system is required. Unless large inocula are used, all fermentation stops or proceeds slowly. The medium supports only a small amount of yeast multiplication and it would be much too expensive to grow a new inoculum for each fermentation. The method of yeast recycling employed at Springfield consisted of pumping the finished beer to a flow splitter, which, by means of constant head pressure and orifices, metered the flow to five 5,000-gal per hour centrifuges. The yeast suspension, comprising 7% of the total volume, was put directly into a fermentor or into a storage tank, and the clarified beer was put into beer wells for distillation.

The maximum number of times the yeast could be recycled was not determined. Different batches of yeast were recycled eighteen times in 70,000-gal fermentations without affecting the fermentation adversely. The fine black precipitate formed in the wort on exposure to the air separated with the yeast and caused the yeast cream to become dark. This had no apparent effect on the yeast activity. Fermentations were carried out by putting the clarified wort into a fermentor, to comply with government regulations, and then adding the yeast cream. The temperature could be adjusted only before delivery to the fermentation section.

Fermentations proceeded normally when 1% yeast by volume was employed. Yeast multiplication was regulated by the addition of urea and phosphate. Addition of 0.02% urea and 0.006% monosodium phosphate by weight was sufficient to maintain this yeast level. Increasing the urea to 0.03% and the phosphate to 0.008% resulted in a 25% increase in yeast volume. No other effect was apparent. Satisfactory fermentations could be obtained with 0.7 to 0.8% yeast, but never with less. In plant-scale fermentations, there was no evidence of improved yield with acclimatization. Yields with new yeast were essentially the same as those with yeast used many times.

The time required for most fermentations with 1% yeast by volume was about 24 hours. The extreme variation in all the operations was from 16 to 40 hours. There were no facilities for controlling the temperature during fermentation. Actually, there was little temperature rise because of the low sugar content and the use of outside fermentors. The fermentation temperatures varied

between 80°F and 90°F, and this temperature range had no apparent effect on the fermentations.

Fermentation efficiencies were found to compare favorably with pilot-plant results. Approximately 78% of the sugar was fermentable and the yield of alcohol based on fermented sugar varied from 44 to 48.5%. Assuming a theoretical yield of 51.1%, fermentation efficiencies of 86 to 95% were obtained.

The fermentation of wood hydrolyzate at the Springfield plant was carried out successfully. The production of sugar, however, presented some problems, none of which was considered fundamental to the process. The sugar-production method is considered practicable and research is continuing on the process. Present research on wood-sugar utilization is directed toward making a concentrate suitable for animal feed or as a source of sugar for fodder-yeast production.

ANALYTICAL METHODS

The control procedures for the fermentation are essentially those employed for other alcoholic fermentations. These included Brix, pH, titrable acidity, yeast volume, yeast viability, and alcohol determinations. Alcohol determinations on unfermented wood sugar solutions show a small negative blank, and determinations are corrected for this. Sugar was estimated as glucose by the Shaffer-Somogyi[103] method. Additional information on the analysis of wood sugar is available.[89]

## Recent European Fermentation Practice

Four plants in Europe produced alcohol from wood hydrolyzates by continuous fermentation with recycled yeast. This was an important advance in the processing of wood sugar and is of general interest.

Continuous fermentation on an industrial scale is a new field. A small amount of information is available in the German literature on the application of this procedure to wood sugar.[26,27,93] The recycling of yeast in the fermentation of sulfite liquor in a Canadian plant[59,60,92] and the continuous fermentation of sulfite liquor in an American plant[19] have been described. (See Chapter 4.) Patents on the continuous fermentation of wood sugar are held by Scholler and coworkers[98,99] and by Karsch.[53] Further information on the German operations was obtained following World War II and has been made available.[9,35,76,90,91]

The techniques used in Europe for the fermentation of Scholler-process wood sugar varies somewhat in the different plants. The newest and most successful of these plants is the one at Ems, Switzerland,[111,112] A more complete description is available of the newest German plant.[35,90] The discussion that follows gives the essential features common to all European operations.

LIQUOR PREPARATION

The hot acid sugar solution, containing about 4% sugar, is neutralized with calcium carbonate and calcium hydroxide and is clarified by filtration[35,90] or by settling and centrifuging.[111,112] The liquor is adjusted to fermentation temperature by vacuum flashing [111,112] or by heat exchangers and atmospheric cooling tower.[35,90] The neutralized solution has a pH of approximately 4.5. Titration of 100 ml of this solution requires 2.25 ml of 1 *N* sodium hydroxide.

FERMENTATION EQUIPMENT

The prepared wood-sugar solution is passed through three to five fermentors connected in series. The total working volume of the fermentors is one-fourth to one-eighth of the daily liquor production and results in a hold-up time of 3 to 5 hours. Each fermentor is equipped with a small stirrer[35,90] or is arranged for bubbling carbon dioxide through the liquid.[111,112] The fermented beer is passed through a bank of centrifuges, where the yeast is recovered and returned to the first fermentor.

ORGANISMS

The culture in use at two German plants was found to be a mixture of *Torulopsis* and other yeast species.[10,35,90] This is a novel culture for this purpose. At another German plant[76] and at the Swiss plant,[111,112] *Saccharomyces* was used. The concentration in the Swiss plant was maintained at 5 g of fresh yeast per 100 ml. This was sufficient to allow a 3- to 4-hour fermentation that was 80% complete in the first hour. Dead yeast accounted for about 30% of the total, but it did not accumulate beyond that level. The yeast growth was regulated by the concentration of the nutrients. At one German plant, the liquor was prepared for fermentation with 0.01 to 0.02% superphosphate and 0.02% ammonium sulfate. When the activity of the yeast fell off, 0.005% of potassium chloride and 0.0025% of magnesium sulfate were used[35] in addition to the before-mentioned nutrients.

CONTAMINATION

Wood-sugar solutions show an unusual resistance to infection

At the German Holzminden plant, wood sugar was brought to fermentation temperature in an ordinary atmospheric cooling tower. In all of these plants, the culture could be recycled for years without replacement. Reports from the Swiss plant show that practically no bacteria are present in the fermented beer and that the pH of the beer is usually within 0.1 pH unit of the feed. Any bacteria that might be present tend to be concentrated in the beer rather than in the recovered yeast cream.

YIELDS

The yield of sugar and alcohol in a Scholler plant was discussed in 1938 by Fritzweiler and Karsch.[27] According to these authors, 100 parts of softwood yield 51 parts of sugar, of which 41.3 parts, or 81%, are fermentable. This portion is converted to alcohol with an 88% fermentation efficiency to yield 23.4 l of alcohol. Plant losses reduce this to 22.7 l as a maximum practicable yield.

Data on the Holzminden plant[35,90] show that the best average yields, obtained during the second year of operation, amounted to 19 l per 100 kg of wood. The operation, however, was undoubtedly hampered by the war. The Swiss plant now produces 21 l of absolute alcohol per 100 kg of wood, and the monthly output is 60% or more above design capacity.

## ECONOMICS OF ALCOHOL FROM WOOD WASTE

The production of alcohol from wood requires a greater investment per gallon than alcohol from any other source with the possible exception of the process based on petroleum gases.[20] This fact requires the plant to have a high capacity in order to keep fixed charges at a minimum. The cost of raw material is low, but it requires much processing and in order to have the same output, a plant must handle more material than in conventional fermentation processes.

The economics of the first wood-sugar process operated in the United States during World War I have been given by Sherrard.[104] In 1921, the production cost of alcohol by this process was estimated at 25¢ per gallon.

Data are not available on the cost of alcohol or sugar production by the Bergius process. The one plant operated in Germany produced only food yeast. The cost data for this, however, is an index of the usefulness of the process since the greatest costs in the process resulted from sugar production. The plant investment alone

amounted to about 60¢ per lb of yeast per year. The fixed charges at 20% of the capital investment amount to 12¢ per lb of yeast produced. When high labor and raw material costs are added to this, the process is obviously of no interest under conditions in the United States.

The Scholler wood-hydrolysis plant at Holzminden, Germany, was erected in 1938 at a cost of 4.4 million marks, or 1,770,000 dollars. The plant employed 155 men and actually produced 3.2 million l or 850,000 gal of alcohol a year.[35,90] The labor required was about 0.4 man-hour per gallon and the plant investment was $2.08 per gal per year. The charges from these two items alone are several times higher than the total production cost for alcohol made from molasses in the United States during this period.

No cost data are available on the alcohol produced in the Ems, Switzerland, plant, but it is known that the production is now 60% above design capacity.[111,112] The Holzminden plant output was about 25% below design capacity.

The economics of the production of alcohol from wood waste in the United States by a percolation process has been discussed by Faith and Hall[20,21] and by Harris and Beglinger.[40] The operation of the Springfield alcohol plant did not provide data adequate for estimating the economics of the process. Using the wood, labor, and chemical costs given by Harris in 1946, the production cost of alcohol at this plant, when operated at design capacity, would be approximately 32¢ per gallon, assuming a 7-year amortization. Expected production costs in the Springfield plant were much lower than those in European plants because of a shorter cycle time and lower labor requirement.

## RESIDUES AND WASTES IN WOOD HYDROLYSIS

In all wood-hydrolysis processes, lignin, amounting to at least 30% of the weight of the wood, remains as an insoluble residue. The only important use for lignin is as a fuel for the plant.

More than 11 lb of lignin are obtained for each gallon of alcohol produced in a wood-hydrolysis process. If this lignin could be sold at a profit of 1¢ per lb, a credit of 11¢ could be applied to the production cost of each gallon of alcohol.

Reducing sugars amount to roughly three-fourths of the organic material in wood hydrolyzate. The reducing sugars are about 80% fermentable by *Saccharomyces*. All of the nonfermentable residue

remains in the stillage and constitutes a problem in waste disposal.

Plants at Ems, Switzerland,[112] and Dessau, Germany, process the still residues to produce a *Torulopsis* fodder yeast. This uses nearly all of the residual reducing material and a significant amount of organic acids.[90] Experiments have been made in this country on processing wood-sugar still residues in a similar manner.[49]

## CONCLUSION

Wood hydrolyzates have been fermented rapidly and efficiently in pilot-plant and industrial-scale operations in several countries. No serious difficulties in the fermentation are encountered when the best techniques available are utilized. The yields of alcohol, based on fermentable sugar present, are similar to those obtained in the fermentation of other carbohydrate raw materials.

## BIBLIOGRAPHY

1. Ant-Wuorinen, O., *Svensk Papperstidn.*, **45**, 149 (1942).
2. Ant-Wuorinen, O., *Suomen Kemistilehti,* **15A**, 31 (1942).
3. Ant-Wuorinen, O., *Chem. Tech.,* **15**, 253 (1942).
4. Armstrong, E. F., and K. F. Armstrong, *The Carbohydrates,* New York, Longmans Green, 1934.
5. Adkins, H., R. L. Frank, and E. S. Bloom, *J. Am. Chem., Soc.,* **63**, 549 (1941).
6. Bausch, H., *Z. angew. Chem.,* **42**, 790 (1929).
7. Bergius, F., *Ind. Eng. Chem.,* **29**, 247 (1937).
8. Boehm, R. M., H. E. Hall, and J. A. MacDonald, U. S. Patent 2,421,985 (1947).
9. Bunker, H. J., Dessauer Werke für Zucker und chemische Industrie A. G., U. S. Dept. Commerce, *Off. Pub. Bd. Rept. PB1095* (1945).
10. Butlin, K. R., Dept. of Scientific and Industrial Research, Teddington, Middlesex, England, Private communication.
11. Cameron, S. H., and G. Borst, *Proc. Am. Soc. Hort. Sci.,* **36**, 255 (1939).
12. Campbell, W. G., *Biochem. J.,* **29**, 1068 (1935); *Nature,* **136**, 299 (1935).
13. Campbell, W. G., and H. J. Bunker, U. S. Dept. Commerce, *Off. Pub. Bd. Rept. PB18897* (1945).
14. Collingsworth, D. R., and J. J. Reid, *J. Bact.,* **30**, 653 (1935).

15. Cooke, L. M., J. L. McCarthy, and H. Hibbert, *J. Am. Chem. Soc.,* **63**, 3056 (1941).
16. Demuth, R. von, *Z. angew. Chem.,* **26**, 786 (1913).
17. Dore, W. H., *Ind. Eng. Chem.,* **12**, 476 (1920).
18. Dunning, J. W., and E. C. Lathrop, *Ind. Eng. Chem.,* **37**, 24 (1945).
19. Ericsson, E. O., *Pulp & Paper Ind.,* **19**, No. 11, 42 (1945).
20. Faith, W. L., *Ind. Eng. Chem.,* **37**, 9 (1945).
21. Faith, W. L., and J. A. Hall, *Chem. Eng. News,* **22**, 525 (1944).
22. Farber, E., German Patent 669,861 (1939).
23. Faucounau, L., *Bull. inst. pin,* 11-23, 70-88 (1934), from Wise.[120]
24. Foth, G., *Handbuch der Spiritusfabrikation,* Berlin, Paul Parey, 1929.
25. Foth, G., *Chem. Ztg.,* **37**, 1221 (1913).
26. Fritzweiler, R., and Rochstroh, *Z. Spiritusind.,* **59**, 229 (1936).
27. Fritzweiler, R., and W. Karsch, *Z. Spiritusind.,* **61**, 207 (1938).
28. Freudenberg, K., *Trans. Faraday Soc.,* **32**, 74 (1936).
29. Freudenberg, K., *Ann. Rev. Biochem.,* Stanford University P. O., Calif., Annual Reviews, Inc., 1939.
30. Freudenberg, K., and G. Blomquist, *Ber.,* **68B**, 2070 (1935).
31. Freudenberg, K., and W. Kuhn, *Ber.,* **65B**, 484 (1932).
32. Freudenberg, K., W. Kuhn, W. Durr, F. Bolz, and G. Steinbrunn, *Ber.,* **63B**, 1510 (1930).
33. Freudenberg, K., and K. Soff, *Ber.,* **66A**, 19 (1933).
34. Gardner, F. E., Calif. Agr. Expt. Sta., *Tech. Paper,* **20**, 42p. (1925).
34a. Gilbert, N., I. A., Hobbs, and J. D. Levene, *Ind. Eng. Chem.,* **44**, 1712 (1952).
35. Greaves, C., *Scholler Wood Sugar Plant at Holzminden,* U. S. Dept. Commerce, *Off. Pub. Bd. Rept. PB18941* (1945).
36. Hägglund, E., *Holzchemie,* 2nd ed., Leipzig, Akademische Verlagsgesellschaft m.B.H., 1939.
37. Hägglund, E., F. Klingstedt, T. Rosenqvist, and H. Urban, *Z. physiol. Chem.,* **177**, 248 (1928).
38. Hägglund, E., *Papierfabr.,* **25**, 52 (1927).
39. Hamilton, H. L., *Influence of Lignin and Related Materials on Physical and Chemical Properties of Soils,* Thesis, University of Wisconsin, 1948.
40. Harris, E. E., and E. Beglinger, *Ind. Eng. Chem.,* **38**, 890 (1946).
41. Harris, E. E., E. Beglinger, G. J. Hajny, and E. C. Sherrard, *Ind. Eng. Chem.,* **37**, 12 (1945).

42. Harris, E. E., J. D'Ianni, and H. Adkins, *J. Am. Chem. Soc.*, **60**, 1467 (1938).
43. Harris, E. E., G. J. Hajny, M. L. Hannan, and S. C. Rogers, *Ind. Eng. Chem.*, **38**, 896 (1946).
44. Harris, E. E., M. L. Hannan, and R. R. Marquardt, *Paper Trade J.*, **125**, No. 22, 34 (1947).
45. Harris, E. E., M. L. Hannan, and R. R. Marquardt, *Ind. Eng. Chem.*, **40**, 2068 (1948).
46. Harris, E. E., M. L. Hannan, R. R. Marquardt, and J. L. Bubl., *Ind. Eng. Chem.*, **40**, 1216 (1948).
47. Harris, E. E., J. F. Saeman and C. B. Bergstrom, *Ind. Eng. Chem.*, **41**, 2063 (1949).
48. Harris, E. E., J. F. Saeman, and E. C. Sherrard, *Ind. Eng. Chem.*, **32**, 440 (1940).
49. Harris, E. E., J. F. Saeman, R. R. Marquardt, M. L. Hannan, and S. C. Rogers, *Ind. Eng. Chem.*, **40**, 1220 (1948).
50. Haworth, W. N., *J. Soc. Dyers Colourists*, 16-23, Jubilee Issue, 1884-1934.
51. Johnson, M. C., and E. E. Harris, *J. Am. Chem. Soc.*, **70**, 2961 (1948).
52. Hawley, L. F., and L. E. Wise, *The Chemistry of Wood*, New York, The Chemical Catalog Co., 1926.
53. Karsch, W., British Patent 480,570 (1938); German Patent 692,812 (1940); U. S. Patent 2,155,134 (1939).
54. Katzen, R., R. S. Aries, and D. F. Othmer, *Ind. Eng. Chem.*, **37**, 442 (1945).
55. Katzen, R., and D. F. Othmer, *Ind. Eng. Chem.*, **34**, 314 (1942).
56. Kerr, R. W., *Chemistry and Industry of Starch*, 2nd ed., New York, Academic Press, 1950.
57. Kressman, F. W., *U. S. Dept. Agr. Bull. No. 983* (1922).
58. Leonard, R. H., and G. J. Hajny, *Ind. Eng. Chem.*, **37**, 390 (1945).
59. Les Usines de Melle, Canadian Patents 341,720 (1934); 348,549 (1935); 402,847 (1942).
60. Les Usines de Melle, French Patent 818,809 (1937); U. S. Patent 2,230,318 (1941).
61. Levy, C., Private communication.
62. Liang, P., *Z. physiol. Chem.*, **244**, 238 (1936).
63. Lüers, H., *Z. angew. Chem.*, **43**, 455 (1930).
64. Lüers, H., *Z. angew. Chem.*, **45**, 369 (1932).
65. Lüers, H., *Holz Roh- u. Werkstoff*, **1**, 35 (1937).

66. Lüers, H., C. Enders and K. Kärnbach, *Biochem. Z.*, **296**, 47 (1938).
67. Lüers, H., G. Fries, W. Hüttinger, E. Mörike, C. Enders, K. Kärnbach, and F. Wieninger, *Z. Spiritusind*, **60**, 7, (1937).
68. Lüers, H., and J. Mengele, *Biochem. Z.* **179**, 238 (1926).
69. Menzinsky, G., *Svensk Papperstidn.*, **45**, 421 (1942).
70. Moelwyn-Hughes, E. A., *Trans. Faraday Soc.*, **25**, 81 (1929).
71. Olson, E. T., R. Katzen, and R. H. Plow, U. S. Patent 2,156,159 (1939).
72. Olson, E. T., and R. H. Plow, U. S. Patent 2,156,160 (1939).
73. Ormondy, W. R., *J. Soc. Chem. Ind.*, **45**, 267 (1925).
74. Ott, E., *Cellulose and Cellulose Derivatives,* New York, Interscience, 1943.
75. Partansky, A. M., U. S. Patent 2,203,360 (1940).
76. Pavcek, P. L., Wood Saccharification by the Scholler Process, Dessauer Zucker Raffinerie, U. S. Dept. Commerce, *Off. Pub. Bd. Rept. No. 4313* (1945).
77. Perlman, D., *Ind. Eng. Chem.*, **36**, 803 (1944).
78. Peterson, W. H., J. F. Snell, and W. C. Frazier, *Ind. Eng. Chem.*, **37**, 30 (1945).
79. Phillips, M., *J. Am. Chem. Soc.*, **51**, 2420 (1929).
80. Phillips, M., and M. J. Goss, *Ind. Eng. Chem.*, **24**, 1436 (1932).
81. Plow, R. H., J. F. Saeman, H. D. Turner, and E. C. Sherrard, *Ind. Eng. Chem.*, **37**, 36 (1945).
82. Reid, J. J., and J. L. Baldwin, *J. Bact.*, **27**, 29 (1934).
83. Ritter, G. J., and L. C. Fleck, *Ind. Eng. Chem.*, **14**, 1050 (1922).
84. Ritter, G. J., and E. F. Kurth, *Ind. Eng. Chem.*, **25**, 1250 (1933).
85. Saeman, J. F., *Ind. Eng. Chem.*, **37**, 43 (1945).
86. Saeman, J. F., *Ind. Eng. Chem., Anal. Ed.*, **19**, 913 (1947).
87. Saeman, J. F., J. L. Bubl, and E. E. Harris, *Ind. Eng. Chem., Anal. Ed.*, **17**, 35 (1945).
88. Saeman, J. F., and E. E. Harris, *J. Am. Chem. Soc.*, **68**, 2507 (1946).
89. Saeman, J. F., E. E. Harris, and A. A. Kline, *Ind. Eng. Chem., Anal. Ed.*, **17**, 95 (1945).
90. Saeman, J. F., E. G. Locke, and G. K. Dickerman, Production of Wood Sugar in Germany and Its Conversion to Yeast and Alcohol, U. S. Dept. Commerce, *Off. Pub. Bd. Rept. No. 7736* (1945).

91. Saeman, J. F., E. G. Locke, and G. K. Dickerman, *Paper Trade J.* **123**, No. 12, 38 (1946).
92. Sankey, C. A., and M. M. Rosten, *Pulp Paper Mag. Can.*, **45**, 171 (1944).
93. Schaal, O., *Cellulosechem.*, **16**, 7 (1935).
94. Scholler, H., *Wochbl. Papierfabrik*, No. 5 (1935).
95. Scholler, H., *Chem. Ztg.*, **63**, 737 (1939).
96. Scholler, H., German Patent 676,967 (1939).
97. Scholler, H., U. S. Patent 2,394,334 (1946).
98. Scholler, H., and M. Seidel, U. S. Patent 2,188,192 (1937).
99. Scholler, H., and R. Eichenmeyer, U. S. Patents 2,083,347 and 2,083,348 (1934).
100. Schorger, A. W., *Ind. Eng. Chem.*, **9**, 748 (1917).
101. Schorger, A. W., *The Chemistry of Cellulose and Wood*, New York, McGraw-Hill, 1926.
102. Schorger, A. W., and D. F. Smith, *Ind. Eng. Chem.*, **8**, 494 (1916).
103. Shaffer, P. A., and M. Somogyi, *J. Biol. Chem.*, **100**, 695 (1933).
104. Sherrard, E. C., *Chemical Age*, **29**, 76 (1921).
105. Sherrard, E. C., *Ind. Eng. Chem.*, **14**, 948 (1922); U. S. Patent 1,454,521 (1923).
106. Sherrard, E. C., "Wood Plastics as Developed at the Forest Products Laboratory and Their Future Significance," Madison, Wisc., U. S. Forest Products Lab., *Mimeo R1209*, 1939.
107. Sherrard, E. C., and G. W. Blanco, *Ind. Eng. Chem.*, **15**, 611 (1923).
108. Sherrard, E. C., and F. W. Kressman, *Ind. Eng. Chem.*, **37**, 5 (1945).
109. Sjolander, N. O., A. F. Langlykke, and W. H. Peterson, *Ind. Eng. Chem.*, **30**, 1251 (1938).
110. Sutermeister, E., *Chemistry of Pulp and Papermaking*, 3rd ed., New York, John Wiley, 1941.
111. Trechsel, M. A., Private communication.
112. Trechsel, M. A., *Neue Züricher Zeitung*, Beilage "Technik," June 12, 1946.
113. Tscherne, M., *Beiträge Biol. Pflanz.*, **24**, 271 (1937).
114. Van Beckum, W. G., and G. J. Ritter, *Paper Trade J.*, **108**, No. 7, 27 (1939).
115. White, E. V., *J. Am. Chem. Soc.*, **63**, 2871 (1941); **64**, 302, 1507, 2838 (1942).
116. Willstätter, R., German Patent 273,800 (1913).
117. Willstätter, R., and L. Zechmeister, *Ber.*, **46B**, 2401 (1913).

118. Winters, R. K., *Forest Products Research Soc. Proc.*, **2**, 15 (1948).
119. Winters, R. K., G. H. Chidester, and J. A. Hall, *Wood Waste in the United States*, Report 4 from a reappraisal of the forest situation, U. S. Dept. Agr. Forest Service, 1947.
120. Wise, L. E., *Wood Chemistry*, New York, Reinhold, 1944.
121. Wise, L. E., and F. C. Peterson, *Ind. Eng. Chem.*, **22**, 362 (1930).
122. Wise, L. E., E. K. Ratliff, and B. L. Browning, *Anal. Chem.*, **20**, 825 (1948).
123. Wolfrom, M. L., and L. W. Georges, *J. Am. Chem. Soc.*, **59**, 282 (1937).
124. Wolfrom, M. L., and J. C. Snowden, *J. Am. Chem. Soc.*, **60**, 3009 (1938).
125. *Wood Products for Fertilizer*, New Haven, Conn., Northwestern Wood Utilization Council, June 29, 1945.

CHAPTER 6

# THE BREWING INDUSTRY

*Robert I. Tenney*

The industry centered around the preparation of beer can truly claim to be the mother of all industrial fermentations. Whenever and wherever man has formed a civilization which left records for later study, we find that he had a fermented beverage. The type of agriculture in which he engaged or contacted determined whether the beverage was of a mead type derived from honey or plant saps, a wine type derived from fruits, or a beer type derived from cereal grains.

Sanskrit writings tell us that the ancient Aryans made a beverage from the fermented juice of the moon plant, called soma, and one produced in larger quantities, called sura, from the fermentation of an infusion of millet and barley. Irrefutable evidence ascribes the preparation of a fermented cereal beverage on a professional and industrial scale to each of the Oriental and African civilizations.

Tradition and mythology claim the gods taught man to make beer to compensate him for the troubles he has to endure. The Egyptians in 2000 B.C. credited their goddess Osiris with these teachings. The Greek historian, Herodotus, wrote about 500 B.C.

crediting brewing to Bacchus. In the middle ages, the German peoples credited a legendary king, Gambrinus, who was supposed to have been a consort of Osiris, with the discovery of beer. The Norse god, Hymer, was keeper of the brew kettle.

Regardless of these legends, the recognition of something worth repeating in the accidental spontaneous fermentation must be accepted. It grew as a household art and, while households became larger and larger as on feudal estates, the need for specialists and industrialization was developing the practice into a well defined art. Success in the art and batch-to-batch quality were primarily dependent on the rigid adherence to recipes, a touch of luck, and an ability to cover up flavor variations by the addition of spices, herbs, and similar materials.

Basically, beer is made today in a manner so similar to that employed by the ancients that we might say the improvements have been those developing when any art accepts the assistance of science and becomes a technology, utilizing the best features of both art and science. Man has learned to describe, define, and measure many of the changes occurring when barley is converted through malt to beer or ale. As each phase of the process becomes well defined, it develops into a science. But so many questions are unanswered as yet that brewing is still an art in many respects.

It was when science was applied to brewing that the other fermentation industries became possible. Chemical science did not advance until relatively recently in comparison to the age of the brewing of beer. Instruments as basic as the thermometer and hydrometer were not used widely until about 1780. The brewing industry was already 5000 years old by then. Pasteur's fundamental study, which established that fermentation is an aspect of the life activities of living yeast cells, was done because a brewery was having difficulty, and his "Etudes sur la Bière" was only published in 1876.

From the time of Pasteur, technical advance was more rapid. Breweries were quick to adopt the machinery which was developed in the 19th century. Steam power, the ice machine, material-handling equipment and power-transmission systems were eagerly applied to the brewers' problems in their early stages of development. Brewing laboratories supplied the equipment for many

fundamental studies. Emil Christian Hansen, Alfred Jorgensen and Pasteur are but a few of the men who contributed to science through brewery association. Arnold[2] has discussed in greater detail the history of beer and brewing.

Today, the industry is conducted on a highly technical plane, utilizing much of the art but embodying new technological developments as rapidly as definitions, equipment, principles and premises are evolved. Several publications[8,12,14,16] are available which describe the modern brewing practice in considerable detail and should be consulted by those who desire more information than is within the scope of this discussion.

All malt beverages, beer, ale, porter, stout, and malt tonics, are made fundamentally in the same manner. Figures 36 and 37 give

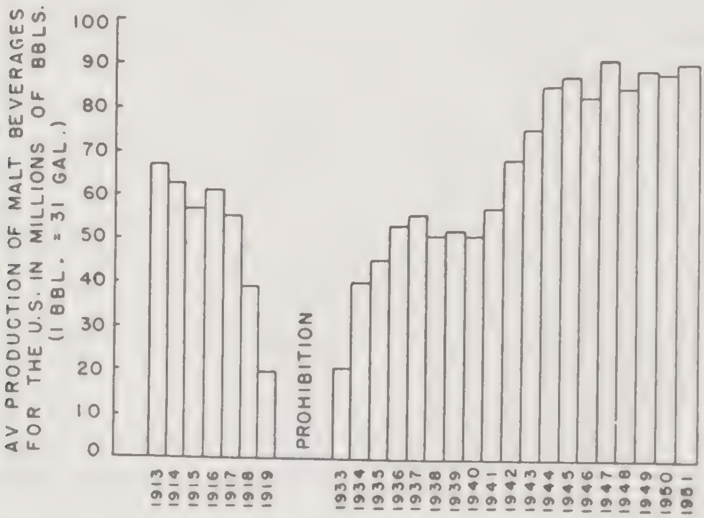


FIGURE 36. *Annual Production of Malt Beverages in the United States*

information on the volume of production of malt beverages in the United States and other countries. If one considers only the brewhouse portion of the processes, various malt extracts and non-alcoholic cereal beverages are also closely related to the malt


FIGURE 37. *Relative Volumes of World Beer Production for the Year 1938*

beverages. These beverages differ in character because of the different amounts and conditions of the materials entering the process. The basic ingredients are malted barley, water, hops, and yeast. Other substances, called adjuncts, are frequently included for modifying the flavor, the stability, the body characteristics, and the physical appearance of the product.

## TYPES OF BEER

It is the manner in which the ingredients are utilized which determines whether the product is offered as beer, ale, or stout, or by other designation. The differences between these varieties are not clear cut and much of the definition depends on the customers' acceptance of the advertised name. The legal definitions of the malt beverages are sufficiently broad to permit some confusion in borderline cases. As a guide, however, a brief description of the main characteristics of the major types will be helpful.

*Lager beer* is the most prevalent type. The term *lager* means "to rest" and it indicates that the fermented beer has aged. The term is applied specifically to beers produced with bottom-fermenting yeasts to distinguish from top-fermentation ales which are frequently called beers in some localities.

*Pilsner beer* is a name indicating the light character of the beers first produced in Pilsen, Bohemia. These beers are light in body and color and usually contain 3.4 to 3.8% alcohol. Most American brands are similar to this type and many include the term "pilsner" in their brand name. The true Pilsner beer is more hop flavored than most American brands.

*Munchner beer* is a name designating the type of beer originally produced in Munich, Germany. It has a fuller malt flavor and darker color than Pilsner types and is sweeter due to the use of less hops. Little of the beer offered in the American market is true to this type as it is more satiating than the public desires, in general.

*Bock beer* is a traditional spring tonic to the beer market. It is customarily brewed in the winter for sale around Easter time. It is considerably darker in color than most other types and is frequently sweeter and heavier bodied. Many stories concern its origin and source. *Bock* is the German word for goat, which is, in turn, the Greek *Aries* or Zodiacal sign for March. This accounts for the association with a goat. Caramelized or roasted malt is used in its production. Originally, and still, it is a festive beer. It is heavier and more expensive to produce than ordinary dark-colored beers which are generally made by adding caramelized malt or sugars before or after fermentation.

*Ale* is a term frequently used interchangeably with beer in some localities. The English type ales are produced by an infusion wort and fermented with top-fermenting yeasts. Those true to type are hopped at relatively high rates and usually obtain further hop contact in the cellars. Ales frequently have higher alcoholic contents up to 8% by volume though this is not a necessary characteristic. Ale of 3.2% alcoholic content is also made. Ales are frequently characterized by a sharper acid bite and a vinous flavor due to higher ester content. Some malt beverages made with bottom-fermenting yeasts are sold and accepted as ales so that little accurate distinction can be made between ale and beer.

*Stock ale*, however, is always a top fermentation product and

has a heavier original extract and a resulting higher alcohol content. It is generally aged 1 or 2 years at temperatures above those employed for aging lager beer. During this time, it acquires a vinous flavor and frequently a musty character. While popular in England and Canada, little stock ale reaches the United States market except as a blending ingredient to flavor regular beers.

*Stout* and *Porter* are typically English. They are top fermented usually from heavy worts produced without malt adjuncts. Alkaline waters are frequently used without neutralization and the resulting products are dark in color and of heavy body. They are not popular selling items in America due to their high alcohol content and satiating effect. In the British Isles, they are frequently sold mixed with thinning ale.

*Kraeusen beer* is any malt beverage that has received a substantial part of its carbon dioxide content through a secondary fermentation which is accomplished after lagering. This is brought about by adding about 10 to 15% of vigorously fermenting wort or sugar solution to the storage beer and permitting the fermentation to be completed in a closed vessel so that the generated carbon dioxide remains in the mixture. Other beers are carbonated by the injection of carbon dioxide gas which is collected for this purpose during the primary fermentation.

## Present-Day American Beers

Despite the advertising claims of the several manufacturers to the contrary, the composition of most beers offered on the market today are remarkably similar. Public acceptance and preference of bland flavor have become reflected in competitive similarity. The beers of the east coast before World War II were substantially heavier in body than those of the west coast. Today local brewers are in direct competition with the large shipping producers and a leveling influence has brought most beers to a common character.

In 1951, six nationally sold beers accounted for almost 27% of the total production. The eighteen brewers producing over 1,000,000 bbls each, sold 48% of the total. In all, 291 companies were operating 331 breweries in the United States as of January 1, 1952. Exclusive of the 3.2% alcohol beers required in some areas and of ales, the average composition of the beer offered on the market in June of 1952 was as follows:

| | |
|---|---|
| Balling | 2.95% |
| Alcohol by weight | 3.58% |
| Extract | 4.71% |
| Original extract | 11.68% |
| Sugar as maltose | 1.12% |
| Sugar degree | 71.0 |
| Attenuation | 60.0 |
| Protein | 0.37% |
| Acidity as lactic | 0.16% |
| pH | 4.4 |
| Color | 2.8 |
| Carbon dioxide volumes | 2.72 |

These figures were compiled by Wahl-Henius Institute from the analyses of seventy-nine different brands. The value of each figure has been weighted with respect to the relative sales volume for each brand to reflect public preference as denoted by sales acceptance. The sample represents 71.7% of the production.

## RAW MATERIALS

*Malt,* within the brewing industry, is always barley malt. While other grains have been and are malted for special purposes, that made from barley is used in all beer. The malting process, which is a vast industry in itself, consists of the following main steps. Cleaned barley is soaked in water to excite growth of the embryo plant. The water is then drained off and the seeds are germinated until the acrospire attains a growth equal to approximately three-fourths of the kernel length. This green malt is then carefully dried to halt the growth and is stored for future use. At one time, practically all brewers germinated their own grain. This process is so unique and distinct from the rest of brewing that specialists have taken over the commercial production of malt for most of the industry. A few brewers in this country and in Europe continue to prepare their own malt because they find it either economical to do so with a view to their volume requirements or because it contributes to the specific character of their product.

During the malting process, the starch of the endorsperm becomes mellow, enzymes are developed, and the flavor and color ingredients so desired by brewers are formed. The utility and character of the malt is brought about by the modification during germination and kilning. Approximately 65 lb of soluble extract

reaches the wort from each 100 lb of malt employed. The reader interested in malting technology is referred to the several texts available concerning this subject, for example that of Hopkins and Krause,[9] and to recent literature.

Malt governs brewing processes primarily because of its (1) starch content as a source of extract, (2) its amylolytic enzymes capable of converting this and other starch into fermentable sugars and nonfermentable dextrins, and (3) its protein content as a source of flavor and yeast nutrients.

*Adjuncts* to malt are chiefly sources of ultimate sugar or extract. They differ in the degree of modification from starch to sugar and in source. Rice and degerminated corn are used in the form of grits or meal. These represent raw starch and will yield close to 75% of the weight as extract to the wort. Refined corn grits are frequently used and, being more nearly pure carbohydrate, will yield about 90% of the weight as extract. All raw starches must be gelatinized by boiling before they may be saccharified by the amylolytic enzymes of the malt.

Prepared starches are also available which have been gelatinized sufficiently to permit attack by the malt enzymes without previous cooking. Typical of this is flaked corn, which is prepared by passing tempered corn-hominy grits through heated pressure rolls so that a thin sheet of gelatinized product is formed.

Still further processing of starch makes its various hydrolysis products available as sources of wort extract. Dextrose sugar is the most refined and is directly fermentable without any further preparation. Sirups, prepared by the corn wet milling industry, contain almost any desired ratio of sugar to dextrin that the brewer may wish.

Adjuncts were introduced because the six-row barleys grown in the United States produced a malt that has enough diastatic activity to convert all of its contained starch and more. The beer made by the incorporation of adjuncts is of paler color than that made from malt alone and contains less protein. Because of this lower protein content, the beer has greater stability and longer shelf life.

*Water* is of considerable importance to a brewer. Depending on the individual plant economies and recirculation practices, 17 to 40 barrels of water are used for each barrel of beer sold. The average is close to 20, of which approximately 2 are used directly in the process and the rest for steam generation, cooling, and washing.

The mineral composition of the water used in the process has a strong influence on the character of the beer produced. High carbonates are responsible for the darker color and heavier flavor of beers made in Munich and much of the character of Dublin's stout. Absence of carbonates, but the presence of calcium sulfate, is responsible for the light beers of Pilsen and the pale ales produced in Burton, England. The paleness of color, lightness of flavor and absence of harshness affected by the Burton water is so desirable that many commercial "Burtonizing salts" have been offered to adjust carbonate waters to a composition approaching that of waters from this English area. It is generally conceded that the most desirable water for brewing has a pH of 6.5 to 7.0 and the following mineral composition: less than 100 ppm of calcium and magnesium carbonates, 250 to 500 ppm of calcium sulfate, and 200 to 300 ppm of sodium chloride.

Traces of magnesium are beneficial to the action of the malt enzymes. Phosphates are also helpful, but iron is undesirable over 1.0 ppm. A good beer can be made from water which differs widely from this composition, but adjustments of processing are usually complicated unless a product of individual character is desired.

*Hops* are the dried strobiles of the female vine *Humulus lupulis.* They are grown extensively in Oregon, Washington, California, and Idaho. The very first use of hops in brewing is unknown, but by 1000 A.D. their use had become rather common. They exert a stabilizing effect on beer and give it an aromatic and pungent character. In the United States, it is customary to use approximately ½ lb of hops per barrel of beer. Ales are hopped at a heavier rate, as much as 2 lb per barrel in some English products.

Hops contain tannin substances which are helpful in the coagulation of protein degradation products from the wort. Their alpha-resins and, to a lesser extent, their beta-resins, exert a preservative effect against many of the gram-positive bacteria. Both contribute a bitter flavor. Gamma-resins are extracted also, but they play no lasting role as they are precipitated again early in the process. Pectin found in the hops is thought to play an important part in the foam retention of the beverage.

## MASHING OR PREPARATION OF WORT

The medium which is fermented to produce beer or ale is called *wort.* This same substance is used without fermentation to produce

malt sirups and similar products. It is prepared by mixing ground barley malt with water and allowing the diastatic enzymes to convert the starch from the malt and that added from adjuncts to soluble sugars and dextrins. This aqueous extract is then separated from the undissolved husk and insoluble protein matter, and it is then boiled with hops for flavor. The process is logically divided into four steps, (1) cooking of adjunct, (2) mashing, (3) separation, and (4) kettle operations.

## Mashing

Three systems are in vogue for conducting the mashing operations. Each has different means to vary the temperatures in order to control the relative quantities of sugar and dextrin formed. This, in turn, determines the amount of alcohol and extract in the finished product. The higher the temperature of conversion the faster is the reaction, but the lower the quantity of sugar formed.

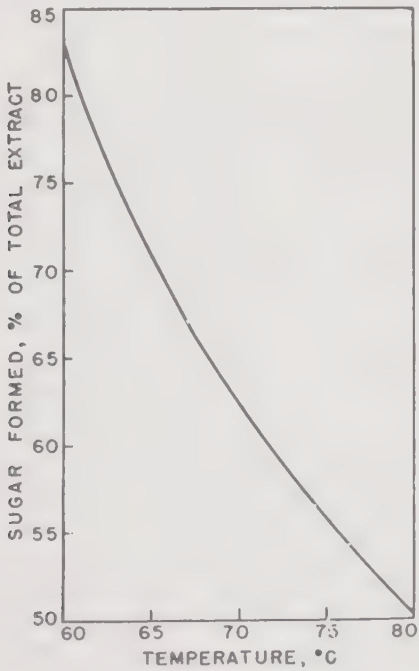


FIGURE 38. *Relationship between the Temperature of Conversion with Malt and the Proportion of Sugar Formed.*

If high alcohol content is desired, conversions are conducted at lower temperatures for longer times.

Single-temperature mashing is the *infusion* process. Figure 38 shows the percentage of sugar formed in the total extract obtained by single-temperature mashing. The time of mashing varies to allow complete conversion of all starch. In the infusion process, the ground malt and water are mixed so as to obtain a selected temperature between 65° and 75°C which is held for approximately 1 hour or until an iodine test shows all starch to be converted. Wort making for beer is always conducted so as to obtain complete saccharification before fermentation begins. In this respect, mashing for beer differs from that of the distiller who does not sterilize his medium before fermentation. Beer wort is sterilized by boiling before the yeast is added and, therefore, no active amylolytic enzymes are carried into the fermentor.

The infusion method is popular in England, in the New England states, and in eastern Canada for the production of ales, but it is seldom used for the production of lager beers or in the rest of the industry.

Continental Europe and areas influenced by the German methods, using only malted grain, have used the *decoction* process. This works at several temperatures to enable enzymic or bacterial action at more nearly optimum conditions. The mash starts out at temperatures near 40°C to take advantage of the proteolytic enzymes of the malt. After a rest of 30 minutes or more, a portion of the mash is withdrawn and boiled in a separate container. This hot mash is then returned to the main portion, thus increasing the temperature of the whole mash. This process may be repeated several times, stopping at various temperatures for the formation of lactic acid (near 50°C); for high sugar content (60 to 68°C); and for rapid completion of the saccharification (close to 75°C).

The use of adjuncts in the United States has led to the development of the *combination* or *cooker* mash which uses steam and the boiling adjunct to accomplish these temperature changes. Figure 39 illustrates a typical mashing schedule for the production of a wort having a sugar degree of approximately 70%. Many modifications of this are possible and are used. The relative amount of sugar and consequently the alcohol content of the beer is regulated by control of the time interval in reaching the main saccharifying period and the temperature of that period. The faster the tem-

perature rise or the higher the conversion temperature, the less sugar and more nonfermentable dextrins will be formed. The rela-

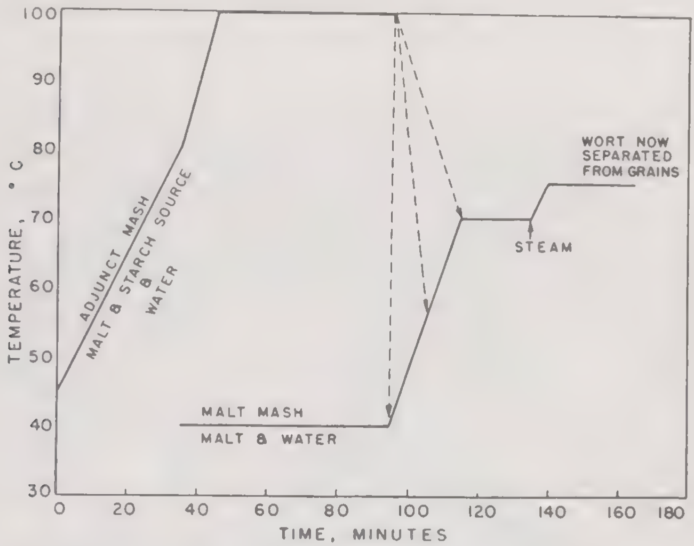


FIGURE 39. *Typical Mashing Schedule for an Adjunct Beer.*

tionship of sugars to nonfermentables is expressed as "sugar degree." This represents the fraction of total extract which is in the form of reducing substances calculated as maltose and expressed as percentage. The alcohol content and beer body are controlled through manipulation of the total extract in the wort and of the sugar degree of that extract.

## Separation

The wort and grain residues may be separated by use of a filter press, centrifuge or, more commonly, a straining tank (lauter tub). At times, the straining is conducted in the same vessel in which the starch conversion takes place. A slotted or perforated false bottom in the tank permits the wort to run out, retaining the grains as a filter bed. Filter presses allow the use of a more finely ground malt, increasing the yield. They are employed in many breweries. Centrifuges have been used in the Scandinavian countries, but have not been adopted in the United States chiefly because the advantages do not outweigh the extra power consumption and

the cost of replacing still serviceable equipment. Brew kettles and straining tanks in a modern brewery are pictured in Figure 40. The separation of the wort distinguishes mashing for beer or ale from mashing for whiskey or alcohol. The next step of wort or media boiling is another difference.

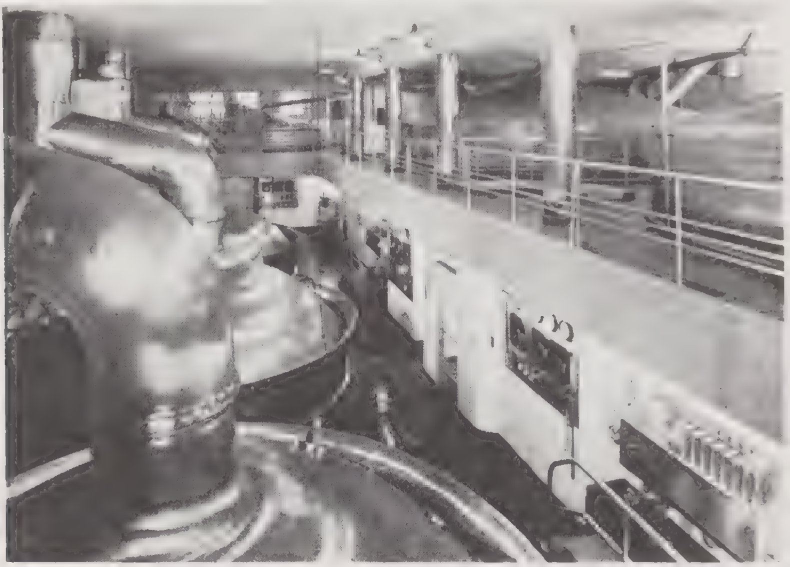

FIGURE 40. *Brew Kettles and Straining Tanks* (Courtesy—Blatz Brewing Co., Milwaukee, Wis.)

### Wort Boiling

Wort is boiled in a vessel equipped with a steam jacket, coils, or a percolator. During this processing step, a portion of the protein, which was hydrolyzed during malting and mashing, is coagulated. This fraction would make the beer turbid if not removed. Some caramel is formed during boiling and the wort becomes both sterilized and concentrated. Hops are added at various stages of the boiling to serve a multiple purpose. They assist in albumin coagulation, act as a preservative against gram-positive bacteria, aid in foam formation, and contribute flavor. The method of blending and adding the hops controls the extraction of the resins and volatile oils. It is subject to limited chemical control and remains a part of the industry's artistry.

After the hops and much of the coagulated substances have been removed by a strainer, the wort is aerated and cooled. A variety of

equipment is used for this in different plants and air-conditioning is common. Double-pipe enclosed heat exchangers, plate coolers and open or Baudelot coolers are all employed for this purpose. Asepsis at this process point is critical and the design is quite an important factor in brewery quality control.

*Wort filtration* has become a rather popular step recently.[7] Albumins, hop resins, and mineral precipitates will settle from cold wort and are frequently eliminated, in part, by pumping the contents of a fermenting tank into another tank the second day of fermentation. Filtration before the yeast is added avoids the necessity for settling, gives a cleaner yeast crop, and facilitates clarification of the beer after fermentation. As frequently practiced, however, danger of infection may be introduced by exposing the wort before and during the filtration without any protection from a vigorous yeast.

Pilot-plant equipment for carrying out all mashing operations of the brewhouse is shown in Figure 41.

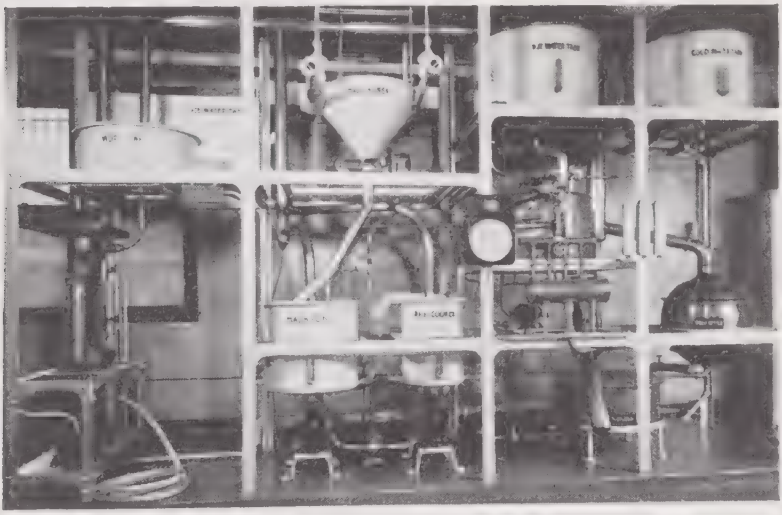

FIGURE 41. *Brewhouse Equipment in an Experimental Brewery* (Courtesy—Wahl-Henius Institute, Chicago, Ill.)

## YEAST CULTURES

Organisms employed in fermentations for the production of malt beverages are various species and strains of true yeasts. They include both top- and bottom-fermenting strains. This distinction

depends on whether the yeast rises to the top or settles to the bottom of the fermenting tank during the active fermentation period. Top yeasts are not normally used except in ale production, but both ale and lager beer are made with bottom yeasts.

Prior to Pasteur and Hansen the yeasts used by brewers had been selected from spontaneous fermentations. By long-continued use and repeated trading between brewers, it is logical to assume that only a few actual varieties were in use. These had largely lost their power to sporulate and were known as "culture" yeasts.

After Hansen's isolations and development of the pure culture technique, a better definition and distinction of strains became possible. The microorganisms of fermentation have been discussed by Jorgensen.[11] Today, several hundred yeast strains suitable for brewing are in the collections maintained by most brewing laboratories, such as Institut für Gärungsgewerbe in Berlin, Carlsberg Laboratories in Copenhagen, and Wahl-Henius Institute in Chicago. These original isolates for plant use are developed in quantities sufficient to inoculate pure culture apparatus. Approximately thirty of the four hundred brewers in the United States operate pure culture equipment of their own. The others secure yeast periodically from those who have the equipment, frequently under the supervision of one of the brewing stations.

One brewer may retain a culture without renewal for several years. Another may change yeast stock every few months. The frequency depends on the vigor imparted to the yeast by the wort fermented and on the amount of contamination.

Since bacterial cells are lighter than yeast cells, they can be effectively washed from a yeast deposit by simple settling procedures. Approximately 3 lb of liquid yeast are recovered from a fermentor for each pound originally added. The yeast for pitching one brew comes from an earlier one. The excess yeast is a valuable by-product which too frequently is discarded.

It is interesting to speculate on the possibilities of hybridizing yeasts to combine optimum fermenting properties with whatever combinations of enzymes are desired. Lindegren[13] (see also Chapter 13 of Volume II) and others have been able to control the enzymic abilities of hybrids produced through stimulation of sexual rather than asexual reproduction. To date, none of these artificial strains has been used commercially by breweries, although their potentialities are recognized.

## CONTAMINATION PROBLEMS

Wort has a natural resistance to many infectious microorganisms. Its low pH (5.0 to 5.4) is too acid for the development of most bacteria and the hop resins exert a preservative action against those which are gram positive. As the yeast rapidly consumes the nutrients, denying them to less vigorous competing organisms, alcohol and carbon dioxide are formed which also exert a preservative effect and the pH drops even lower (4.2 to 4.8).

In spite of the natural resistance, it is essential to practice strict sanitation. Lactic and acetic acid bacteria are frequent airborne contaminants and, if permitted to gain too much activity, will impair the flavor of the beer. Most of these are rod shaped, but several strains of cocci are also encountered, among them forms which frequently appear as tetrads and are known erroneously to the brewer as "sarcina."

Regular microscopic examinations are made of the yeast crop to guide selection of that to be used for pitching fresh wort. It is desirable to maintain the total bacteria count of the yeast crop at less than 1%.

Beer, after fermentation, is subject to attack by foreign organisms, such as molds and mycoderma, but these are easily controlled by normal sanitary measures. Since beer is generally kept under an atmosphere of carbon dioxide during aging and since the yeast has already depleted the oxygen supply, aerobic organisms develop very slowly. Shimwell[15] has found that anaerobes which grow under the conditions prevailing during aging have caused contamination in beer. Virus and 'phage infections have not been found in beer.

## FERMENTATION

In the production of American lager beer the aerated wort is cooled to 46° to 52°F and yeast is added as the wort is distributed into the starting or settling tanks. From ¾ to 1 pound of liquid yeast is added per barrel of wort. The standard brewery barrel is 31 gal. In general, higher wort-extract contents, lower fermentation temperatures, and less vigorous yeasts require addition of larger quantities of seed yeast than low gravity worts, high fermentation temperatures and vigorous yeasts.

The fermentation room of a modern brewery is shown in Figure 42.

FIGURE 42. *Brewery Fermentation Room* (Courtesy–Miller Brewing Co.)

Within 24 hours after pitching, a foam ring appears at the edge of the tank and gradually spreads to cover the entire surface.

The extract content will drop during this initial stage by about 0.5% and the temperature will normally increase about 2°F. At this point, when the liberation of carbon dioxide keeps the vigorous yeast in suspension, the fermenting wort is transferred to another tank. Thus dead and weak yeast cells, precipitated proteins, and insoluble hop resins are left behind either as a deposit on the tank bottom or trapped in the dense foam which is left behind. Cold wort filtration eliminates the need for this transfer, but as additional aeration is also given by this pumping over, it is frequently useful even though the wort has been filtered.

The foam cover thickens as fermentation proceeds until, in 40 to 60 hours, it becomes a dense and viscous mass on the surface. At this stage, it resembles the texture of cauliflower and is known as "young kraeusen." During the third or fourth day, the foam cover reaches its maximum depth of 8 to 12 in. and becomes somewhat lighter in texture. At this stage, the development of new yeast cells is proceeding most rapidly as are also the increase in temperature and decrease in extract.

Usually by the fifth day, this kraeusen begins to collapse be-

cause the rate of gas evolution is insufficient to support the foam. By then, the evolution of heat is too slow to counteract the refrigerating effect of the cold room in which the tank is held and the temperature drops. From 7 to 9 days after the yeast was added to the fermentor, activity has all but subsided and the yeast settles to the bottom of the tank, leaving a relatively clear and black-appearing liquid at the top of the tank. Intensive cooling is usually applied at this stage over a period of 1 or 2 days to hasten this settling.

During the liberation of gas, a yellowish or brownish deposit collects in the center of the kraeusen head. This "nest" consists of oxidized hop resins which are less soluble due to reduction of the sugar content and lower pH. This is usually skimmed off to prevent a harsh, bitter flavor. Closed fermentors frequently have foam-collecting cones or chambers for removal of this precipitate.[6]

The fermentation is controlled by the proper selection of the starting temperature and by the use of coils in which a cooling

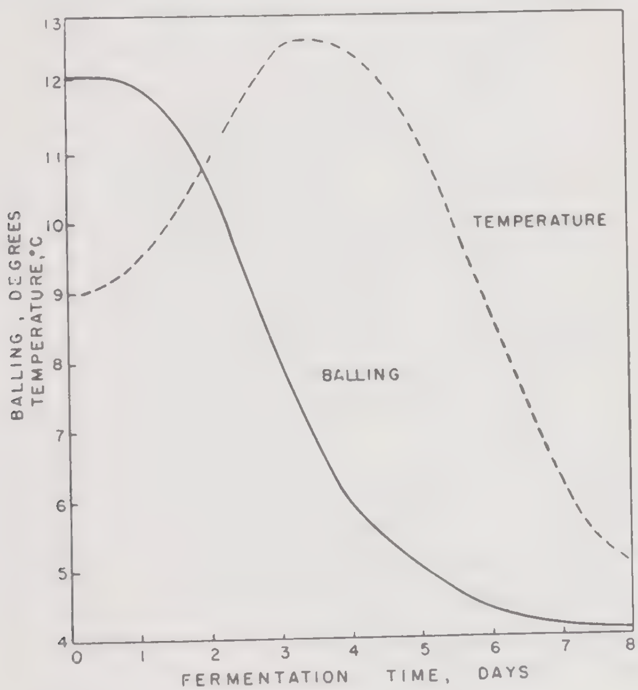


FIGURE 43. *Temperature and Balling Changes in a Typical Lager Fermentation*

medium is circulated. Brine and sweet water are generally employed for this purpose. The fermentation of 1 lb of sugar generates about 260 Btu of heat. In general, the higher the temperature is permitted to rise the quicker the fermentation will be complete and the faster will be the drop in extract content. There is a slight difference between the rate of the various side reactions and that of the main fermentation with respect to temperature. This results in the development of different flavors in beers which are fermented from identical worts at different temperatures. Composition of wort, time and temperature of fermentation, and subsequent cellar treatment are the main controls the master brewer has over the flavor of his product.

The exact conduct of a fermentation will depend on individual plant conditions and preferences, but the course of a typical one is shown in Figure 43. The data were compiled during the preparation of a typical adjunct lager brew in the experimental brewery of Wahl-Henius Institute and no attemperation was applied to demonstrate the full effect of internal development of heat. The beer was in a wooden tank in a room held at 40°F.

## MATURING AND CELLAR TREATMENT

After the beer is fermented, it is held in "ruh" tanks for several weeks. During this time, the beer is clarified by the sedimentation of coagulated nitrogenous substances, insoluble phosphates, and yeast. Filtration with diatomaceous filter aids expedites this step and pulp filters complete the production of a brilliant beer. At times, clarifying agents of a gelatinous nature are added to the beer in the tanks to facilitate clarification. The storage corridor in a modern brewery is shown in Figure 44.

Chillproofing is the protection of the beer against the development of turbidity on exposure to cold. The Wallerstein[17] patents are typical and were the first in the application of enzymes for this purpose. The process consists of adding proteolytic enzymes which will reduce the molecular size of the residual protein hydrolyzate to insure increased solubility. The Ash,[3] and Joachim[10] patents cover typical procedures for removal of these hydrolyzates by precipitation or adsorption. In either case, the object is to remove nitrogenous bodies larger than peptones and proteoses.

Since beer is subject to flavor changes on oxidation, antioxidants have been added for many years. Sulfites have been used

FIGURE 44. *Storage Corridor in a Brewery* (Courtesy—Miller Brewing Co.)

extensively for this purpose although not to as great an extent as in wines. Wines may contain as much as 250 ppm of sulfur dioxide. Beers are flavored too highly if such high levels are used and the Food and Drug Administration does not permit over 25 ppm of sulfur dioxide to be used in the United States. Higher contents are permitted in other countries, but flavor is usually noticeably affected. Ascorbic acid[5] has been used as an antioxidant for beers and will possibly gain in favor as this compound is produced more inexpensively.

Carbon dioxide is added to the beer at the end of the aging process either by injection of the gas or by the admixture of freshly fermenting beer. After adjustment of the gas content to a definite value between 2.5 and 3.0 volumes, the beer is given a final polishing filtration and is transferred to a bottling tank.

## PACKAGING

The greater bulk of beer is packaged in bottles or cans and is pasteurized. Draught or keg beer is not pasteurized in the United

States, but it is in Mexico, Scandinavian countries, and Continental Europe. Every effort is made to eliminate air from the final package.

## KEEPING QUALITIES

Unpasteurized beer will, of course, spoil due to development of microorganisms within a relatively short period, depending on the refrigeration it receives. Sterile filtration has been accomplished by the use of Seitz and similar filters, but the combination of sterile product and sterile package has not been achieved in this country on a large enough scale to make possible a regular supply of unpasteurized beer. Impairment of foam quality, the lack of completely sterile filling conditions, and the preferences of the consuming public have stood in the way of the acceptance of this procedure.

Even beer that has been pasteurized is perishable and subject to many influences that degrade its quality as it remains unconsumed after it has left the brewery. Sunlight, excessive heating, agitation, and internal oxidation give rise to changes in flavor and brilliance which become more noticeable as the beer gets older. While a beer which has been bottled several months may yet remain quite palatable, it will not compare with the same beer in a fresh condition.

"Preservative" agents have been used with little success in an effort to avoid the change in flavor that results from pasteurization. Benzoates and salicylates are deleterious to foam. Propionates introduce undesirable odors and flavors. Chloroacetates have found the most favor, but they are far from ideal. At best, preservatives can only retard microbial development and should not be used to mask an infected product. No preservatives are actually employed in the brewing industry in the United States. The Food and Drug Administration does not permit the use of chloroacetates and similar agents because they are harmful and are not necessary in good manufacturing procedures. Pasteurization is accepted as good practice, but does introduce undesirable flavor changes.

## ANALYTICAL METHODS AND LITERATURE

In an industry as old as brewing, it is only natural that the literature on analytical methods is voluminous. Many of the basic techniques of analytical chemistry were developed in brewing laboratories as answers to observed deviations were sought. In an

effort to reach closer agreement between different laboratories and to establish common methods of analysis, the American Society of Brewing Chemists was founded in 1935 as an outgrowth of work done on malt analyses beginning in 1934. This society has published valuable tables and a manual of methods.[3] The methods approved by this society have been largely adopted also by the Association of Official Agricultural Chemists.[4] Those particularly interested in details of analysis of brewing raw materials and products are referred to the publications of these societies.

Journals dealing with brewing were among the first of the technical publications. Typical important journals are listed in Table 27. The list is far from complete and is intended only to show the early start and some of the publications in which original articles appear.

Table 27. SELECTED TECHNICAL BREWING JOURNALS

| Journal | Publication dates | Country of origin |
|---|---|---|
| American Brewer | 1867–current | U.S.A. |
| American Brewers Review | 1887–1918 | U.S.A. |
| Annales des fermentations | 1935–current | France |
| Brewers Digest | 1926–current | U.S.A. |
| Comptes rendus laboratorie Carlsberg | 1921–current | Denmark |
| Journal of the Institute of Brewing | 1887–current | England |
| Petit journal du brasseur | 1897–current | France |
| Proceedings, American Society of Brewing Chemists | 1939–current | U.S.A. |
| Transactions, American Society of Brewing Technology | 1910–1918 | U.S.A. |
| Wallerstein Laboratories Communications | 1938–current | U.S.A. |
| Wochenschrift für Brauerei | 1883–1944 | Germany |

Several trade papers, having articles of technical value, are published in many countries. Modern Brewery Age, Brewers Journal, Journal of the Incorporated Brewers Guild and Brewing Trade Review are in the English language and are worthy of mention. The first two are published in the United States and the last in England. A complete listing is beyond the scope of this discussion.

## BY-PRODUCTS

*Extracted grains* which are separated from the wort are valuable as stock feed. As obtained from the brewhouse operations, this residue contains about 80% moisture. Much of it is sold in this wet condition to farmers for immediate feeding, but the bulk is dried to a moisture content of approximately 10% so that it may be held without spoilage. These dried brewers' grains contain 12 to 15% protein, 3 to 5% crude fat, about 60% total carbohydrates, with 20% crude fiber. They serve as an excellent source for vitamins of the B complex.

*Surplus yeast* was, at one time, almost universally discarded into the sewers, but is now gathered for further processing into a variety of substances. Brewers' yeast is rich in water-soluble vitamins of the B complex and in ergosterol and it is now used as human food and animal feed, as a source of vitamin concentrates, and for obtaining other biochemical products.

*Carbon dioxide* is generated during the fermentation in excess of the requirements for the carbonation of the finished beer. In many plants, a portion of the excess is collected for counter-pressure purposes, and some sell their surplus gas to other users.

## BIBLIOGRAPHY

1. American Society of Brewing Chemists, *Tables Related to Determinations on Wort and Beer; Tables for Extract Determinations in Malt and Cereals; Methods of Analysis,* Sturgeon Bay, Wisc., American Society of Brewing Chemists, 1949.
2. Arnold, J. P., *Origin and History of Beer and Brewing,* Chicago, Wahl-Henius Institute, 1911.
3. Ash, C., U. S. Patent 1,234,255 (1917).
4. Association of Official Agricultural Chemists, *Official and Tentative Methods of Analysis,* 6th ed., Washington, D. C., Association of Official Agricultural Chemists, 1945.
5. Gray, P. P., and I. Stone, *Wallerstein Lab. Commun.,* **2**, No. 6, 32 (1939); U. S. Patent 2,159,985 (1939).
6. Gull, R., U. S. Patent 2,136,685 (1938).
7. Harrison, E. W., *Am. Brewer,* **80**, No. 6, 21 (1947).
8. Hind, H. L., *Brewing Science and Practice,* New York, John Wiley, 1940.

9. Hopkins, R. H., and C. B. Krause, *Biochemistry Applied to Malting and Brewing,* New York, D. Van Nostrand, 1937.
10. Joachim, H., U. S. Patent 2,416,007 (1947).
11. Jorgensen, A., (revised by A. Hansen), *Microorganisms and Fermentations,* London, Charles Griffin, 1948.
12. Kloss, C. A., *The Art and Science of Brewing,* London, Stuart & Richards, 1950.
13. Lindegren, C. C., *Wallerstein Lab. Commun.,* **7**, 153 (1944).
14. Master Brewers Association of America, *The Practical Brewer,* Winnetka, Ill., Master Brewers Association of America, 1946.
15. Shimwell, J. L., *J. Inst. Brewing,* **43**, 507 (1937).
16. Wahl-Henius Institute, *American Handybook of the Brewing, Malting and Auxiliary Trades,* Chicago, Wahl-Henius Institute, 1908.
17. Wallerstein, L., U. S. Patents 995,820 (1911); 2,077,447; 2,077,448; 2,077,449 (1937).

CHAPTER 7

# COMMERCIAL PRODUCTION OF TABLE AND DESSERT WINES

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A wide variety of alcoholic beverages can be and has been produced by the fermentation of various fruits, berries, herbs, starchy plants or plant parts, honeys and, even milk (both animal milk and coconut milk), as well as cereals. The term "wine," however, is restricted by custom to alcoholic beverages made by fermentation of fruits or berries or their juice and other suitable agricultural products, such as honey, rhubarb, etc. Grape wines, however, constitute by far the greatest proportion of alcoholic fruit beverages of commerce (see Table 28) and wine, as defined by the various governmental agencies concerned with its production, is essentially the fermented alcoholic beverage produced from grape juice. Wines other than from "the juice of sound, ripe grapes" are always required to show their source on label declaration. Blackberry wine and apple wine are produced in larger amounts in the United States than the other fruit and berry wines, which include apricot wines, berry wines (elderberries, loganberries, raspberries, strawberries, etc.), and wines made from cantaloupe, citrus fruit, cherries, papaya, pears, plums, and dried fruits (dates, figs, prunes, raisins). Grapes have been used for wine making longer than any other fruit and are the only fruit systematically selected and grown

TABLE 28. FRUITS USED IN COMMERCIAL PRODUCTION OF WINES AND BRANDIES IN THE UNITED STATES.

| Fruit | Tonnage used[a] |
|---|---|
| Grapes | 1,657,555 |
| Raisins | 55,444[b] |
| Apples | 10,536 |
| Peaches | 5,894 |
| Berries | 4,514[c] |
| Plums | 3,994 |
| Pears | 3,599 |
| Cherries | 1,229 |
| Others | 15,714[d] |

[a] Quantities crushed or pressed and deposited in fermentors for production of wine and brandy from July 1, 1946 to June 30, 1947.

[b] Converted into fresh tons from dried pounds.

[c] Includes blackberries, elderberries, gooseberries, loganberries, raspberries, strawberries, youngberries, and other berries.

[d] Includes apricots, currants, dandelions, dates, figs, grapefruit, honey, mixed fruits, nectarines, oranges and prunes.

Source: *Wine Institute Bulletin*, **374**, April 2, 1948.

in large quantities for use in wine making. It is for this reason that pleasing, palatable wines can be made from selected grapes without such amelioration as is necessary for the production of most other fruit wines.

The commercial production of wine in the United States is small in comparison with the 6 billion gallons of world production. Production in Europe and French Africa combined accounts normally for over 90% of the total output; French territory alone produces nearly half of the world total, followed by Italy and Spain. Portugal, Greece, the Balkan States, Germany, Chile, Argentina, Australia and South Africa also produce considerable quantities of wine. California produces over 80% of the wine made in the United States, reaching in 1946 a record high production of over 177,000,000 gal. The other two principal wine producing regions are: Louisiana, Arkansas, and Missouri; and New York, Ohio, New Jersey, and Michigan. New York State leads in the production of sparkling wines.

The production of wine in the United States is under the jurisdiction of the Federal Alcohol and Tobacco Tax Unit of the Bureau of Internal Revenue, the Federal Food and Drug Administration, and the State Food and Drug Administrations. The manu-

facture of wines containing over 14% alcohol, particularly as it involves changes in volume or alcoholic content (production and use of wine spirits), is more closely regulated and restricted than of wines whose alcoholic contents are derived solely by fermentation.

The wines produced in California are made from about one hundred and twenty-five varieties of the cultivated species, *Vitis vinifera,* which is the species cultivated in the vineyards of Europe bordering the Mediterranean Sea, in Asia, South America, South Africa, and Australia. When grown in the various viticultural areas of California defined by climatic conditions, the species matures with sufficient sugar content to allow the production of wine by the partial or complete fermentation of the juice of grapes, with or without the addition of diluted or undiluted grape concentrate, and with or without addition after fermentation of pure condensed grape must or grape spirits. The addition of sugar is expressly forbidden by state law in California.

In the Eastern wine producing regions, varieties of the native American grape, *Vitis labrusca,* are used and because of their high acid and low sugar content, it is necessary to add both sugar and water. The addition of sugar and water, however, is limited to an increase in volume of not over 35%; the added sugar may not exceed 11% by weight of the wine. The resultant wine must contain at least 5 parts per thousand of acid before fermentation and not more than 13% of alcohol after complete fermentation.

## TYPES OF GRAPE WINE

The many types of wine that are produced differ in relative sweetness, alcoholic content, and carbon dioxide content, depending on the methods of production. The variety, maturity, climate and soil conditions under which the grapes are grown also influence the character of wine. Color, relative sweetness, alcoholic content, presence of effervescence, the viticultural area of origin, and the dominant grape variety are the general class characteristics used in the classification of wines. The most important natural classification, on the basis of use, is into two large groups, table wines and dessert or appetizer wines. *Table wines* contain less than 14% of alcohol by volume and are served with various courses at dinner. They may be "red" or "white" in color and may contain carbon dioxide gas produced by secondary fermentation (sparkling wines). *Dessert wines* contain over 14% alcohol, usually about 20%, and

commonly contain some sugar so that they are popularly known as "sweet" wines in contradistinction to table wines, which are usually, but not always, free of fermentable sugar and are called "dry" wines. The terms "dry" and "sweet" are confusing, because the so-called "dry" wines are sometimes sweet to the taste and the "sweet" wines sometimes dry. The alcoholic content of dessert wines is raised during production by addition of grape spirits or brandy, after suitable preliminary fermentation to check the fermentation and assist in the retention of natural grape sugars in the wine. Their period of fermentation is shorter than that for table wines and, after fortification, they are less subject to bacterial spoilage during aging and storage.

Within each of these two classes of wines, a number of types are recognized in the industry, although their nomenclature has not been as highly standardized in the United States as in Europe where the viticultural area of origin is carefully delineated and where the particular varieties used in a given viticultural area for a given type of wine are restricted. Both nonvarietal and varietal names are used in California and elsewhere in the United States.

The most important red table wines are: *Burgundy,* a heavy-bodied, dark-colored, dry, red table wine; *Claret,* a red wine of no predominant characteristic flavor and aroma which is lower in extract and color than Burgundy; *Chianti,* a fairly heavy, moderately astringent red wine; *Cabernet, Barbera* and *Zinfandel* which derive their names from the grape variety from which they are predominantly made in California. In Eastern United States, small quantities of varietal red table wines are made from Ives, Concord, or Norton grapes. The most important white table wines are Rhine wines *(Moselle, Riesling, Traminer, Hock); Chablis* and *White Chianti,* and the natural sweet wines, Dry, Sweet and Chateau *Sauternes.* The two most important classes of sparkling wines are *Champagne* and *Sparkling Burgundy.*

The California dessert wines, with the exception of *Angelica* which is a native name, and *Muscatel* which is derived from a varietal name, are named after European wines which they do not resemble closely. The red, sweet dessert wines are represented by the deep red colored *Port* or the more amber tinted *Tawny Port.* The white, sweet dessert wines are represented by: *Angelica,* an amber or yellow colored sweet wine without muscat flavor; *Muscatel,* a similar wine with pronounced muscat flavor; *White Port,* a water-

white, sweet wine without muscat flavor; *Sherry,* a sweet wine with rancio, or cooked or caramel flavor below 7% sugar; and *Tokay,* a rancio-flavored sweet wine with a pink tint. *Madeira, Marsala* and similar wines of commerce are amber-colored, rancio-flavored, sweet wines of poorly defined types. Ports and Sherries are produced also in Eastern United States from labrusca varieties of grapes. The former has a readily detectable, foxy flavor, characteristic of the Concord grape, but this flavor is removed from the white varieties used for Sherry making by special treatment.

The industrial practices developed in California for the production of basically clean, sound wines of standard quality, which form the bulk of the wines of commerce here as well as abroad, will be presented in this chapter as they apply to the current practices in larger wineries. The fine wines which make up a small part of the production in California as well as in the world, are made with great care from selected grapes, frequently aged for considerable periods, and are the high-priced wines on which a reputation for quality in wine making is built. The basic processes and unit operations applied in the commercial production of the more important California wines are presented in some detail, together with a brief discussion of the principles involved. Further details, both on principles and practices, may be found in the selected list of references to American, French, German, Italian and Spanish treatises on wine making, listed at the end of this chapter.

## SELECTION AND HANDLING OF GRAPES

Wine grapes must have the proper composition and character for the type of wine to be produced and must be sound, mature, and fresh. The suitability of a grape variety for a particular type of wine depends not only on its flavoring ingredients but also on the proper balance between the components of its must. The best grapes for the standard dry table wines should yield musts of over 18° but less than 23° Balling; they should have a must acidity of not less than 0.65%, and a pH below 3.3. The natural sweet types of table wines require musts with a Balling degree of 24 to 28; this is necessary to maintain residual sugar in them, yet their acidity should be about 0.60%. Grapes for dessert wines should have a Balling over 24° but should not be allowed to remain on the vines until they have become raisined or have lost their fruitiness. They

should be of moderate acidity, from 0.4 to 0.6% in the finished wine; their pH value may be as high as 4.0.

The maturity of the grapes at harvest and the environmental conditions under which they are grown affect composition. Grapes grown under cool conditions ripen more slowly, retain a higher degree of acidity at maturity, and have a lower pH. The red grapes contain more coloring matter and their anthocyanin pigments are more stable when grown under cool conditions. These conditions favor the production of musts well suited for table wines. Under relatively warm ripening conditions, high sugar and moderate acidity suitable for dessert wines are secured.

California has a wide range of climatic conditions and wine-grape adaptation to the climatic regions has been intensively investigated from 1860 to the present. Production factors, such as scion-stock interrelationships, inherent vigor of the vine, resistance to frost, insect pests and diseases, yield, and composition of the grapes under various soil and climatic conditions have been evaluated. Maturation without undue rotting, sunburning or raisining; type of clusters (tightness, readiness to shatter, and size); thickness of skin; and ease with which the stems and berries can be separated by mechanical stemmers, are some of the factors that have been considered. The time of maturation is an important factor; early-ripening varieties mature in the warmest part of the growing season so that ripening changes proceed too rapidly; late ripening varieties may be spoiled by early rains; varieties that mature in midseason are best for they may be crushed when temperatures are lower. On the basis of these studies, varieties for planting in the five temperature regions of California have been selected. Table wines of best quality are produced in the cool and moderately cool coastal valleys, best natural sweet wines in the warm coastal valleys, and the best dessert wines in the moderately hot and hot interior districts of Northern, Central, and Southern valleys.

## Composition of Grapes

The grapes must have the proper composition and character for the type of wine to be made. The ingredients of the grapes not only affect the flavor of the wine by directly contributing the coloring matters, tannins, acids, flavoring constituents, and sugars for sweetness, but also influence the course of alcoholic fermentation and the type of by-products formed. The physical and chemical

composition of grapes at maturity and the changes in composition during growth, fermentation, and aging have been studied in some detail abroad and to some extent here. The stems on which the berries are borne in most varieties constitute from 2 to 4% of the total weight and contain appreciable amounts of tannins and other extractives whose presence is undesirable in the wine. The fleshy pericarp, known as pulp, surrounded by skins and in which the seeds are imbedded is the greater portion of the fruit itself. From 84 to 95% of the crushed stemmed grapes (the mixture of crushed grapes and juice is referred to in the industry as must) is juice. The skins account for 5 to 12% of the weight and the seeds for 0 to 4%. The outer layers of the fruit (skin mainly) contain the greater portion of the aromatic, coloring and flavoring constituents. The natural tannins are concentrated in the seeds which also contain important amounts of oils and resinous matter. The exact composition of grapes is not known, but it is known that several carbohydrates are present, the two most important being dextrose and levulose. During maturation, the levulose content rises and the levulose-dextrose ratio increases to about unity. Grapes also contain small quantities of pentoses, inositol, pectins, and pentosans.

The chief organic acids present are malic and tartaric, with smaller amounts of citric acid. During ripening, the malic acid content decreases and the total tartaric acid content increases. The free malic and tartaric acids gradually decrease while the acid tartrate content increases to the point where crystalline deposits of potassium acid tartrate form in the cells, just underneath the skin. The pulp of the grape near the skin contains more sugar and less total acid than that near the seeds. The nitrogenous ingredients are chiefly present as basic amino acids, peptides, and purines, with smaller amounts of ammonium compounds and nitrates. The anthocyanin pigments, largely oenin, occur in the skin cells of red grapes. Potassium, sodium, calcium, and iron phosphates, sulfates, and chlorides account for most of the 0.2 to 0.6% ash. The flavoring constituents, but for the methylanthranilate which predominates in the labrusca varieties (Power and Chestnut,[29] Sale and Wilson[31]), the volatile ingredients of Zinfandel grapes and wine (Haagen-Smit, Hirosawa and Wang[17]) and of one Swiss white wine (Hennig and Villforth[18]) are unknown.

The course of the fermentation is affected most by the sugars, acids, and tannins and these should be properly balanced. This

balance is best obtained by selecting suitable varieties and harvesting them at the proper stage of maturity. Musts that are deficient in acid content should be acidified by addition of tartaric acid or citric acid. Proper adjustment of acidity will insure that the fermentation takes place under conditions favorable to yeast and will reduce the susceptibility of the wine to bacterial spoilage and to metallic casse. Fornachon[14] suggested that the pH of the must should be adjusted below 3.6 to avoid a too sour taste of the resulting wine. The titratable acidity rather than pH is a better indication of sour taste, but other factors (sugar content, etc.) also intervene. The addition of tannin (about 1 g per 1) is also beneficial in checking bacterial growth. It is particularly desirable for stabilizing the anthocyanin pigments and for other reasons. The addition of tannin is usually not necessary in making red wines since the skins and seeds form a ready source of tannins. The concentration of sugar is adjusted to suit the type of wine produced. In no case should it be over 28% for, above this concentration, sugar will retard fermentation. Deficiencies in other ingredients necessary to successful alcoholic fermentation are rare in California grapes. They usually contain sufficient potassium, phosphate, and ammonium- or other assimilable nitrogenous-compounds to support growth and activity of yeast. Occasionally, however, musts are encountered which are difficult to ferment dry and which respond to ammonium salts. Since the ammonium ion appears to promote fermentation, deficiencies in nitrogenous substances are best made up by ammonium salts, particularly ammonium phosphate. In the fermentation of fruit juices, honeys, and similar products, addition of ammonium phosphate is necessary.

## Harvesting and Transportation

For the proper operation of a winery, it is necessary to contract for the varieties of grapes, depending on the type of wine to be made, and to arrange for their delivery over the fermenting season at the rate at which optimum operating conditions can be maintained. The yield of grapes per acre varies from about 1 to 3 tons for the finer varieties grown in the north coastal valleys to as high as 16 tons for the common varieties grown in the lower central valley. For ordinary wines, all the crop is harvested at a single picking; the very green, diseased, badly raisined, decayed, or moldy grapes, which may spoil a good wine, are eliminated from the good clusters.

For fine wines, several pickings should be made to get the fruit uniform and all at the best possible stage of maturity. The grapes are usually picked into field lug boxes and hauled in them, or in bulk loads, to the winery. The field lugs are 8 × 14 × 24 in. (inside), holding 40 to 50 lb per box. On the basis of an average weight of contents of 43 lb per box, the packed density is about 23.0 lb per cu ft of space. The clusters are removed from the vines by cutting with curved knives, shears, or snapping the bunches off where the peduncle is crisp enough to allow this. Trained pickers can harvest 10 to 12 boxes a day in the poorer-producing areas to over 30 boxes in high-producing areas. The cost of harvesting varies from about $9 per ton in the coastal valleys to $3 per ton in the southern interior valleys.

Where grapes are delivered to the winery in the field boxes in which they are picked, the trucks are loaded in the vineyard. The filled boxes are brought to the end of the aisles in the vineyards by sleds and tractors or by vineyard trailers and tractors and loaded on the trucks at the ends of the rows. It takes two men about 3 hours to load a truck, as it is driven through the vineyard, with 16 to 18 tons of grapes.

To reduce the cost of loading and unloading trucks and to reduce investment in field boxes tied up during transportation, gondola trucks, introduced by the larger wineries, are widely used for the bulk hauling of grapes. Where gondola trucks are employed, the field boxes are brought to one side of the vineyard and loaded into the gondola by automatic grape loaders. The boxes are brought by belt conveyors, equipped with rubber cleats, to the top of the truck and dumped. By the use of these loaders, a 20-ton-capacity gondola can be loaded in an hour by two men. Two types of gondolas are used, hand and automatically unloading. The first are usually straight-sided tanks about 8 ft wide, 12 ft long, and 2 ft deep; they can be unloaded with an eight- or ten-prong fork at the rate of 10 tons per man hour. A truck and trailer can haul two such gondola tanks. The automatically dumping gondolas are constructed as four-section tanks of about 8 × 6 × 2.5 ft. A tank of this size can hold as much as 6 tons of grapes, equivalent to 100 lb per cu ft in comparison with 23.0 lb per cu ft of field-box space. The gondola tank trucks properly used are more economical means of transportation, can be washed and sterilized more readily than the wooden field boxes, and, for short hauls, they are just as satis-

factory. Their use has been criticized because there is more crushing and damaging of grapes during loading and hauling. Since broken or crushed grapes spoil quickly and may contaminate the wine, bulk hauling for long distances is undesirable. The use of dirty, juice-soaked boxes is also objectionable.

The best practice is to crush the grapes and pump the must into the fermentation vats as soon as possible. Usually, the average distance of haul is between 10 and 20 mi, the average time of hauling is about an hour, and the grapes are unloaded and crushed within hours of picking. Hauling for as long as 4 hours where delivery times are controlled is not detrimental, but where the grapes are hauled over longer distances and are received at the winery the day after picking, their quality for wine making will be reduced, particularly in warm weather. Some of the larger wineries have been successful in crushing the grapes in the field, loading the crushed grapes into gondola trucks, sulfiting, and hauling the sulfited must to the winery for fermentation.

On arrival at the winery, the trucks of grapes are weighed, usually on a 60-ft platform scale, and weighed again when unloaded. Where grapes are purchased at the winery on a sugar basis, a representative sample is taken by the state inspector stationed at the scales (random sample from load delivered in field boxes, or by a sampling tube thrust through the load), crushed, and pressed through a muslin bag or cheesecloth. The extracted juice is poured into a hydrometer cylinder and its Balling reading taken. It is difficult to properly sample the grapes and to obtain a juice sample which is representative of the sugars present in the harder or partially dried berries.

Further details on the production, harvesting, and selection of grape varieties for wine making in California are given by Jacob,[20] and by Amerine and Winkler.[4,5] The wine-production problems of California grapes are discussed by Amerine.[2]

## ALCOHOLIC FERMENTATION

In alcoholic fermentation of sugars, the chief products, alcohol and carbon dioxide, are obtained in essentially equimolecular proportions. The glucose molecule passes anaerobically through twelve stable intermediary steps before becoming alcohol and carbon dioxide; at least three and possibly eight dissociable organic enzymes, twenty or more enzyme proteins, and several inorganic

catalysts (ammonium, potassium, manganese, magnesium, and copper ions) must be provided by the yeast cell. The mechanism of alcoholic fermentation is outlined in Figure 1 of Chapter 2. In addition to alcohol and carbon dioxide, acetaldehyde, glycerol, 2,3-butylene glycol, lactic acid, succinic acid, and citric acid are constant products of alcoholic fermentation and contribute to the flavor and aroma acquired by the wine during fermentation. Esters, chiefly ethyl acetate, are also formed and esterification of the fixed acids, such as tartaric and malic acids, is catalyzed by yeast enzymes. The presence of levulose and dextrose alters the course of fermentation, since the usual strains of wine yeast ferment dextrose more rapidly so that the dextrose-levulose ratio decreases during fermentation. Yeast also attacks the organic acids of grapes and significant changes occur in the composition of these acidic ingredients during fermentation. Higher alcohols and acids are produced in small amounts by the action of yeast on the amino acids present in the must.

The course of alcoholic fermentation, particularly the formation of by-products from carbohydrate and noncarbohydrate sources, depends on the strain of yeast used, the composition of the must (particularly its sugar and tannin content), the temperature at which the fermentation is conducted, the extent of aeration, the amount of sulfite added to the must, and other factors. The nature and concentration of these by-products cannot be predicted accurately. In recent investigations of the conditions governing the accumulation of the major by-products, it has been found that the various substances are related to glycerol production approximately as follows:[16]

5 moles succinic acid + 2 moles acetic acid + 1 mole butylene glycol + 2 moles acetylmethylcarbinol + 1 mole acetaldehyde = 0.9 mole glycerol

About 2.5 to 3.0% of the sugar transformed is converted into glycerol, 0.2 to 0.4% into lactic acid, 0.02 to 0.10% into succinic acid, 0.2 to 0.7% into acetic acid, 0.05 to 0.10% into butylene glycol, and about 1 to 2% is utilized by yeast for growth and respiration.

On the assumption that 1 mole of dextrose is converted into 2 moles of ethyl alcohol and 2 moles of carbon dioxide, the weight

of alcohol produced should be 51.1% by weight of the sugar consumed. Under closely controlled experimental conditions, the highest yields of alcohol obtained rarely have exceeded 48.0% of the sugar converted and lower yields are obtained industrially. The French enologists commonly assume that 1% of alcohol by volume is obtained per 17 g of sugar fermented per l, equivalent to about a 43% conversion by weight. The alcohol content as percentage by volume of wine is usually estimated in terms of the Balling degree of the must by multiplying it by a suitable factor. The factor to be used will vary with the content of nonsugar, soluble solids and with the accuracy with which the must is sampled. It is difficult to obtain a sample of the must which truly represents all the fermentable sugars present and the nonsugar solids are known to vary in amount and composition with grape variety and maturity. The factor found industrially thus varies from about 51 to 57.5% of the Balling degree of the must, but this cannot be used as a criterion of fermentation efficiency. The true fermentation efficiency is not known.

The concentration of alcohol produced from grape juice by a given strain of yeast, the degree to which the sugar content is attenuated, the rate and efficiency of fermentation, and the nature and concentration of by-products are influenced largely by temperature, extent of aeration, sugar concentration, and acidity. Temperature is extremely important; the lower the temperature the higher is the yield of alcohol in fermentation, not only because the fermentation is more complete but also because of the lower loss of alcohol by evaporation and entrainment by the escaping carbon dioxide gas. Temperature also affects the rate of fermentation and the nature and amounts of by-products formed. The optimum temperature of fermentation for most varieties of wine yeasts is 80° to 85°F, although cold-tolerant yeasts are known which will ferment grape juice at 50°F. Above 85°F, the fermentative activity of yeast decreases and fermentations usually cease at 100°F. An increase in temperature decreases the alcohol-tolerance of yeast and increases rapidly the toxicity of acetic acid. More bouquet is formed in a wine by a long, slow fermentation at low temperature than by a short, rapid fermentation at higher temperature. A wine obtained by cool fermentation is easier to clear and is less susceptible to bacterial spoilage. For these reasons, the temperature during fermentation is maintained at not above 75°F for white wines, nor

above 85°F for red wines. The present trend in the industry is toward cool fermentations at about 50°F, particularly for white wines.

The aeration necessary for growth and multiplication of yeasts is obtained ordinarily by crushing and stemming grapes and pumping the must into the fermentors. Yeast multiplies vigorously in such a must, until most of the dissolved oxygen is consumed, and then the yeast ferments the must. Aeration is seldom used during fermentation, except when it becomes sluggish. Excessive aeration during fermentation not only results in a decreased fermentation efficiency but also produces a flat, oxidized wine of poor color and flavor. Aeration favors the formation of acetic acid, succinic acid, and aldehydes.

High sugar content favors low fermentation efficiency and if the sugar content is over 30%, sufficient alcohol and other products are formed to arrest fermentation before all the sugar has fermented. Low acid content favors production of acetaldehyde, glycerol, and volatile and fixed acids, but gives lower yield of aromatic principles. Sulfur dioxide appreciably increases the amount of aldehydes and glycerol. In table wines, especially, great care should be taken to prevent the accumulation of aldehydes and an excessive amount of volatile acids because of their detrimental effect on flavor, color, and stability.

## YEAST CULTURES AND STARTERS

Selected cultures of yeasts are commonly used in wine making, particularly with sulfur dioxide to control fermentation. The wine yeast, *Saccharomyces cerevisiae* var. *ellipsoideus,* is differentiated from the beer yeast, *Saccharomyces cerevisiae,* largely on the basis of cell shape and inability to ferment melibiose. Different strains of wine yeast are identified industrially on the basis of the by-products, differences in fermentation rate, fermentation efficiencies, and sedimentation rate. Important differences occur in their ability to utilize organic acids and other ingredients. In the districts of Europe where wine making has been practiced for centuries, wine yeasts particularly adapted to bringing out the best qualities of the variety of grape grown are probably available but systematic investigations of the effect of various strains of yeast singly and in mixed cultures on the wine produced from various varieties of wine grapes have not been made.

Under natural conditions, no single variety of yeast, but rather a mixture of several varieties, differing in alcoholic, ester, and extract-forming powers, together with certain types of acid-tolerant bacteria, will enter into fermentation. When these are present in proper proportions, under suitable conditions, a mixed fermentation occurs that is responsible for the occasional production of excellent wine by natural fermentation. Because of lack of knowledge about the relation of the associative and competitive effects of mixed cultures on the quality of wine fermentations, the difficulty of obtaining the required flora, and the danger of spoilage in natural fermentations, only selected varieties of wine yeast have been used in the industry. These yeasts have been selected largely on the basis of their ability to form alcohol rather than flavor and are usually imported from select enological regions abroad. It is now known, however, that, in general, flavor formation by yeasts varies inversely with their alcohol-forming powers and that ester formation in wines may occur through the direct agency of yeasts and bacteria. The application of this knowledge to California conditions is yet in its infancy and because of the danger of spoiling wines, mixed cultures are not recommended.

Not all bacteria in wines and musts are harmful; some produce desirable changes. For example, lactic-acid bacteria capable of converting malic acid into lactic acid, with a resultant decrease by about one-third of the total acidity and a marked increase in pH, are especially cultivated in German and Swiss wines of unusually high acid content and the Bordeaux school of enologists considers this so-called malo-lactic fermentation to be a most important factor in determining the quality of their red wines.

The relative desirability of conducting fermentations with a single strain of selected wine yeast *(S. cerevisiae* var. *ellipsoideus)* in comparison with fermentations conducted by mixed flora has long interested European enologists. While it is likely that the proper succession of fermentation flora may be involved in the production of fine wines, there is little scientific evidence on the subject. The possibility of controlling flavor by the use of species of yeasts other than *S. cerevisiae* has particularly attracted the attention of Castelli.[10] He has pointed out that *Torulaspora rosei,* which has been found to be widely distributed in Italian wines, can ferment must with the production of over 10% alcohol, small amounts of volatile acids, and no acetoin and may be more desirable

than the true wine yeast. A systematic investigation of these fields would be highly desirable.

Burgundy and champagne strains of wine yeasts used in California are considered best for starters, because they form a heavy granular and compact sediment toward the end of the fermentation and produce satisfactory fermentations. Wines made with them clear rapidly. Yeasts are generally supplied to the winery as streak cultures on nutrient agar in cotton-stoppered test tubes or bottles. For propagation at the winery, the bottle culture is preferable because of its larger size.

The yeast starter is increased in volume before use, preferably by growing in a suitable pure-culture system. It may be increased in volume, with suitable precautions against infection, by successive transfers from the original culture to quantities of sterile must increasing in volume. For example, starting with a ½-pt culture, successive transfers may be into 1 gal, 5 gal, 50 gal, and 500 gal of sterile must. The transfers are made at the height of activity. The starter is at its maximum activity when the Balling degree of the must in which it is grown has been reduced about one-half. The smaller quantities of must are bulk-pasteurized and the larger (over 50 gal) are sulfited before use. The sulfited must should stand for several hours before the yeast starter is added. In larger containers, aeration may be necessary before or after addition of starter.

A better procedure is to use a pure-yeast-propagating apparatus which consists essentially of two closed tanks equipped with steam and cooling coils and air distributors. One tank is set above the other. Grape juice is introduced into the upper tank, pasteurized, cooled, aerated, and then dropped into several gallons of active starter in the lower tank. When the must is in active fermentation in the lower tank, all but a few gallons of it are withdrawn and fresh sterile must is then introduced into the culture vessel.

It is best to inoculate each fermenting vat with a fresh yeast starter, because transfer from vat to vat is sure to cause contamination with other yeasts. As little as 1% by volume of active starter is satisfactory when the juice is clean and is fairly free from undesirable wild yeasts and bacteria; rarely is it necessary to add over 3% starter. Too much starter should not be used, for it may produce rapid and violent fermentation, during which heat may be generated too rapidly to be controlled, even by cooling.

## CONTROL OF FERMENTATION

The appearance, flavor, and aroma of the wine, its soundness and freedom from disorder or diseases, and its resistance to bacterial attack during storage will depend largely on the kind of microbial growth in the fermenting mass and on the character of the fermentation. The growth and activity of the desired organisms, wine yeasts, must be furthered, whereas that of the undesirable organisms, wild yeasts and bacteria, must be hindered. Fortunately, microorganisms differ in their nutritional requirements, their requirements for oxygen, their tolerance for acid and alcohol, in their resistance to antiseptic agents, such as sulfur dioxide, and in their response to temperature.

The most serious spoilage is caused in musts by the unchecked activity of acetic and lactic acid bacteria and in wines by the activity of lactic acid bacteria. The susceptibility of wine to bacterial attack varies with acidity, alcohol content, presence of substances liberated by decomposing yeast cells, and degree of exhaustion of nutrients, such as phosphates, and nitrogenous matters. Wines from musts of moderate to high acid content, fermented rapidly and continuously at low temperatures, are most resistant to bacterial attack and are, besides, of better quality. For propagating the desired alcoholic fermentation, the number of true wine yeasts should predominate over those of other microorganisms and the environmental conditions (composition of must, temperature, aeration) should be made favorable to their growth and activity.

In the industrial control of fermentation, mass inoculation with active starter into must previously sulfited to inhibit growth of undesirable yeasts and bacteria and temperature control are used.

### Sulfur Dioxide

Sulfur dioxide, a gas at ordinary temperatures and pressures, is widely used as a liquid under pressure or as sulfurous acid or its acid salts (the bisulfites and metabisulfites) in preparing and preserving wine. In water solutions, it exists as dissolved gas, sulfurous acid, bisulfite ion, and sulfite ion. The pH, temperature, and concentration determine the proportions of the species present. When added as a salt (potassium bisulfite, $KHSO_3$, or potassium metabisulfite, $K_2S_2O_5$) to a must, the salt is largely hydrolyzed into sulfurous acid and bisulfite ions. Sulfurous acid and the bisulfites are readily oxidized to sulfates by oxygen or other oxidizing agents

and will also combine with aldehydes, ketones, and sugars to form bisulfite addition products. The pH, sugar content, aldehyde content, and temperature largely influence the proportions of the free and combined sulfur dioxides. The free sulfur dioxide (including dissolved sulfur dioxide, sulfurous acid, and bisulfite ion) exerts the desired effects in wine making and wine preservation. Its utility is based on its reducing or antioxidant properties and its selective antiseptic effect. It also exerts a clarifying, dissolving, and acidifying influence.

The use of excessive amounts of sulfur dioxide should be avoided, because it not only detracts from the flavor of the wine and interferes with the natural aging but also leads to undesirable turbidities and deposits when copper salts are present in wine. Only the minimum quantity necessary for proper control and fermentation should be used. *When proper sanitary precautions are used in picking, crushing, fermenting, and storage, sulfur dioxide need not be used in objectionable amounts.* Small doses repeated as necessary are preferable to a single large dose.

The antiseptic power of sulfur dioxide depends on the composition of the must, particularly its content of substances capable of forming bisulfite addition compounds (aldehydes, ketones, sugars, etc.), pH, and temperature. Sulfur dioxide added to must combines rapidly with aldehydes and sugars and, in the combined form, is markedly less antiseptic. Cruess[11] and Bioletti and Cruess[8] reported it to be one-sixtieth as antiseptic in the combined form toward yeast and acetic bacteria and more recently Ingram[19] found only the free sulfur dioxide to be germicidal to *Zygosaccharomyces* in orange-juice concentrate. Fornachon[14] found, however, that bound sulfur dioxide has a pronounced antiseptic effect on spoilage bacteria in fortified wine and prevents their growth even when very little free sulfur dioxide is present.

The proportion of combined sulfur dioxide increases with sugar and aldehyde content; it decreases with increase in amount of sulfur dioxide added and with increases in temperature and acidity of the must. At a pH of 2.8, in aqueous solutions, about 10% of the sulfurous acid is free, whereas at pH 3.8, it is only 1% free. Moreau and Vinet[27] reported a loss in antiseptic power of 37.6% when the acidity of an acid must, containing 260 mg of free sulfur dioxide per liter, was reduced by 0.3% as tartaric. During the period of 1928 to 1938, the conditions in wine and must were

studied that determine the equilibrium between free and combined sulfur dioxide and an antiseptic index for French wines and musts was set up. No complete investigation of the sulfur dioxide equilibria in California wines and musts has been made.

The antiseptic power of sulfur dioxide also varies with the kind and activity of microorganisms present. Thus, wine yeasts are less sensitive to sulfur dioxide than are most of the common yeasts, molds, and bacteria occurring in grapes and wine. Very small amounts of sulfur dioxide (equivalent to 5 oz of potassium metabisulfite per ton of grapes, in most cases) suffice to prevent growth of molds and wild yeasts. As Cruess[11] and others have shown, 100 ppm of sulfur dioxide (6 oz of potassium metabisulfite per ton) will eliminate over 99.9% of the active cells of microorganisms from normal musts. By properly timing the sulfiting and the addition of wine-yeast starter, the full effect of the maximum amount of free sulfur dioxide is exerted on the injurious organisms, while the wine yeast is exposed to the minimum amount of free sulfur dioxide. Furthermore, the wine yeasts can adapt themselves to sulfur dioxide and become comparatively resistant to it. Porchet[28] has found this adaptation to be quite marked.

The antiseptic action of sulfur dioxide toward microorganisms, particularly yeasts, varies with the stage of development and the numbers, being greater on the resting or sporulating yeasts and the more effective the lower the numbers present. Yeast in full activity is more resistant to sulfur dioxide, partly because of its rapid fixation by the aldehydes formed in fermentation, partly because of the mechanical entrainment of sulfur dioxide gas by carbon dioxide gas, and partly because of the natural increase in resistance of the cell.

The lower the temperature of fermentation, the lower concentration of sulfur dioxide is required to prevent the development of undesirable microorganisms.

To control fermentation, the amount of sulfur dioxide to be added to a given must depends on the degree of ripeness and soundness of the grapes, the temperature of the grapes and must, and the weather conditions at the time of crushing. Overripe grapes, rich in sugar, of high pH, and low in acid, moldy grapes, and warm grapes require more sulfur dioxide than cool, sound grapes of moderate sugar, low pH and high acid content. In Europe,

the amount usually added in hot regions is 100 to 200 ppm; in cool regions less than 100 ppm.

Liquid sulfur dioxide, the gas liquefied under pressure and held in heavy-walled steel cylinders, is now extensively used. Its advantages are purity and relative cheapness. By any of several measuring devices for dispensing the sulfur dioxide, an exact amount of the gas can be introduced directly into the must or wine from the cylinder. Willson, Walker, Mars, and Rinelli[36] described the available methods of measuring sulfur dioxide by weighing, by volume and by metering. The most commonly used method in the winery is a pressure vessel fitted with a gage glass calibrated in volumes equivalent to a pound and usually of 9 to 23 lb capacity so that they can be easily transported.

## Control of Temperature

Considerable heat is generated during alcoholic fermentation and, unless dissipated, will cause a rise in temperature of the must. The amount liberated in the fermentation has been calculated from the difference of heats of combustion for the fermented material and for the products formed. This computation is not very accurate, because there are errors in combustion-heat determinations, because corrections must be made for heats of solutions of products and for escaping gases, because the concentration of reacting substances continuously decreases while that of the products increases, and because the fermentation occurs in successive stages of decomposition of sugar.

Rahn[30] has calculated the heat evolved from 180 g of sugar consumed in the reaction

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2$$

to be 26.0 Cal when the sugar, alcohol, and carbon dioxide are in their standard state; other reported calculations vary from 22 to 33. Genevois,[15] in a more recent calculation, has reported 28.0 Cal when the reacting substances and products are in dilute solution. Winzler and Baumberger[37] have given a calculated value of 22.5 Cal for dextrose at a concentration of $1 \times 10^{-4}$ *M*, alcohol at $2 \times 10^{-3}$ *M*, and carbon dioxide gas, at a pressure of 0.0003 atm.

The heat evolved in actual fermentations has been measured by a number of investigators. Of the earlier measurements, those of Bouffard[9] in 1895 are most consistent; he found the heat of fer-

mentation of grape juice to vary from 23.4 to 23.7 Cal per 180 g of sugar fermented.

On the basis of 23.5 Cal per 180 g of sugar, the temperature of a must containing 22% of sugar would rise about 52°F if all the heat developed by fermentation were prevented from escaping. That is, for each Balling degree of sugar in the must, sufficient heat is generated during fermentation to raise the temperature approximately 2.34°F. If the initial temperature of the must were 60°F, it would reach 100°F and fermentation would cease while still containing 5% of sugar. In practice, these temperatures are not reached because heat is lost by radiation from the open surface of the fermentor to the surroundings, by conduction through the walls of the fermentor, and by loss in the evolved carbon dioxide gas.

The temperature to which the fermenting grapes or must will rise is determined by their temperature when crushed, plus the rise in temperature due to the heat generated by fermentation and minus the heat lost during fermentation by radiation and conduction. The warmer the grapes and the more sugar they contain, therefore, the higher the temperature will rise. Cooling the grapes or must before fermentation, fermenting in vats with a large radiating surface per unit volume, and cooling the fermenting must itself are the means by which dangerously high temperatures can be prevented. Even in the cool coastal regions, where small fermentation vats are used, cooling during fermentation is necessary in controlling the temperature, particularly early in the vintage season. Where large fermenting vats of 3,000 to 10,000 gal capacity are used for fermenting dry table wines, the fermenting must has to be cooled artificially, even if the grapes are cool when crushed.

Cooling must not be too extreme and should not be carried out in the late stages of fermentation, or fermentation will be too prolonged. It is better to cool the must initially, if necessary, to a point some 10°F below the maximum temperature desired and then to cool again at the height of the fermentation, when the Balling degree has decreased to approximately half its initial value and the yeast cells are more numerous and more active. At this stage, cooling will have less retarding effect on the fermentation. The temperature of the must at this stage should be reduced about 1.5°F for each degree Balling, so that the final temperature will not exceed that at this point. It is desirable to cool even further so that the maximum desirable temperature is not exceeded.

A uniform, low fermentation temperature is best and can sometimes be obtained by means of cooling coils through which cold water or other refrigerating fluid circulates. These coils must be properly placed and be large enough for adequate temperature control. More positive results are obtained by passing the must through tubular or plate heat interchangers in which it is cooled by indirect contact with a suitable refrigering agent, such as cool well water, ice water, cold brine, or some other suitable substance. It may be sprayed over the coils through which the must is pumped, or conveyed in outer tubes past inner tubes containing the must. Any refrigerator used should be constructed of corrosion-resistant metals. To facilitate pumping through the refrigerator, the must should be drawn off through a screen, to separate out skins and seeds into a sump. It may then be sent through the cooler. In emergencies, cooling may be accomplished by adding ice directly to the fermentor, but as this dilutes the must, only small quantities should be used.

The use of a properly designed cooling coil mounted within the fermentor will result in a more nearly uniform temperature of fermentation, lower cooling load and less oxidation. Marsh[23] has suggested to mount the cooling coils 1 ft away from the side walls and in central position with respect to the height of the side wall. Soft drawn, 2-in. copper tubes, mounted at 10 to 12-in. centers vertically are most commonly used in concrete fermentors; at least one lineal foot of piping for each 100 gal of fermentor capacity is provided.

In the preprohibition era, this use of cooling coils in red-wine fermentors was not satisfactory, because the centrally located coils and their supporting frames, as installed in wooden fermentors, interfered with the punching down of the cap and did not control the temperature of the cap. Their present successful use in concrete fermentors is due to improved design and the substitution of pumping over for punching in controlling the cap.

The fermentation of red wine in the presence of grape skins, widely used for color extraction, has long been criticized. As early as 1913, Mathieu recommended heating of freshly crushed grapes to a high temperature (140°F) to extract color with subsequent cooling and pressing of the heated grapes and fermentation of the expressed juice at low temperatures. This method was used, to a limited extent, in California during the preprohibition period, but was abandoned later. Most of the red wine made in New York is

made by this process essentially and Marsh[24] has called it to the attention of California wine makers.

Marsh[23] has calculated that the minimum cooling requirements for fermentation are 150,000 Btu per 1,000 gal, with a safe figure of 250,000 Btu for load calculations. The principal external coolers, in most common use by wineries producing table wines exclusively, are single-pass, water-cooled, counterflow, shell and multitube coolers. They consist of several 20-ft lengths of 3-in. or 4-in. galvanized iron pipe inside of which are mounted three to seven thin-walled ¾-in. or 1-in. copper tubes joined together by a system of return bends designed to produce separate water and wine flow. The cooling surface varies from 121 to 128 sq ft in the various types of units. Plate coolers, which are coming into use, have the advantage of flexibility of operation, high rate of heat transfer, small size, and construction of corrosion-resistant metals. Their chief disadvantage is that the must has to be screened carefully to prevent seeds and skin particles from plugging the channels between the plates. The rate of heat transfer in the shell and tube coolers was found by Marsh to vary from 111 to 390 Btu per hour per square foot of cooling area per degree mean temperature difference. The use of cooling water is most economical when the flow of wine and water through the unit are approximately equal.

The temperatures reached in the fermenting vats should be noted frequently. The rise is greater in open red-wine fermentors than in closed white-wine fermentors, because heat is lost more slowly from the surface of the first; in the second, the pomace tends to rise to the top, forming a semidry "cap," which retards heat loss by radiation. One should take the temperature of the wine just below the cap after punching. The heat is highest here because of the insulating effect of the cap. The reading should be taken on a long-stemmed, easily read thermometer the bulb of which is immersed in the vat. Temperatures read on thermometers attached to hydrometers or on small thermometers pulled out of the vat to be read may be low by as much as 10°F. Removing a sample from the vat with a wine thief, transferring it to a hydrometer jar, reading the hydrometer, and then reading the thermometer in the hydrometer is not the proper way to take the temperature of wine. Long-stemmed thermometers with an indicating dial are available for winery use. In the larger plants, recording thermometers are

useful, since they furnish a permanent record of the temperatures attained in fermentation.

Cold weather during the late fall or early winter may result in sticking (cessation of fermentation) because of lowered temperature. In this case, in order to complete the fermentation, it may be necessary to warm the wine by using a tubular heat interchanger, such as a pasteurizer.

## FERMENTATION PRACTICES

There are wide differences in the actual methods of fermentation used in various wineries, depending on the types of wine to be produced, scale of operation, and available equipment. There is even variation in the unit processes involved. It is generally agreed, however, that the most important unit operations are: unloading of grapes, conveyance to crushers, crushing and separation of stems from skins and pulp, conveyance of pulp to fermentation vats, addition of sulfite, addition of pure yeast starter, fermentation, and separation of wine from pomace. The operations in the manufacture of wine are summarized in the flow diagram of Figure 45.

In the production of red wines, color extraction is an additional operation which may be accomplished either by fermentation in contact with skins, or the anthocyanin pigments may be extracted by heat and the colored juice separated from the skins and seeds, cooled, and then fermented. The first process is usually followed in California; the second, and more preferable process, has long been used in New York.

In the production of white wines, contact of the juice with skins and seeds is limited and the juice is separated shortly after sulfiting. Storage of the must after sulfiting is practiced to facilitate separation by action of naturally occurring pectic enzymes and hemicelluloses. The addition of pectic enzyme preparations to the must from white grapes facilitates the separation of skins and seeds from the juice and aids in the clarification of the wine, but may increase the susceptibility to browning or discoloration.

Not only should the grapes brought to the winery be in sound condition, but all equipment (conveyors, crushers, pumps, must and wine conduits, tanks, etc.) coming into contact with the grapes or wine must be clean and, where possible, adequately sulfited. The pick-up of metals, such as copper and iron, particularly by the wine should be avoided. The tolerance of wine for iron salts is

known to be greater than for copper salts. A wine containing over 5 ppm total iron or over 0.2 ppm copper is unstable and is subject to clouding and sedimentation after bottling. The more heavily sulfited white wines, particularly, should not come into contact with copper-bearing surfaces. Excessive aeration during fermentation, and especially during the handling of wine in the usual cellar operations, should be prevented in order to avoid browning and discoloration.

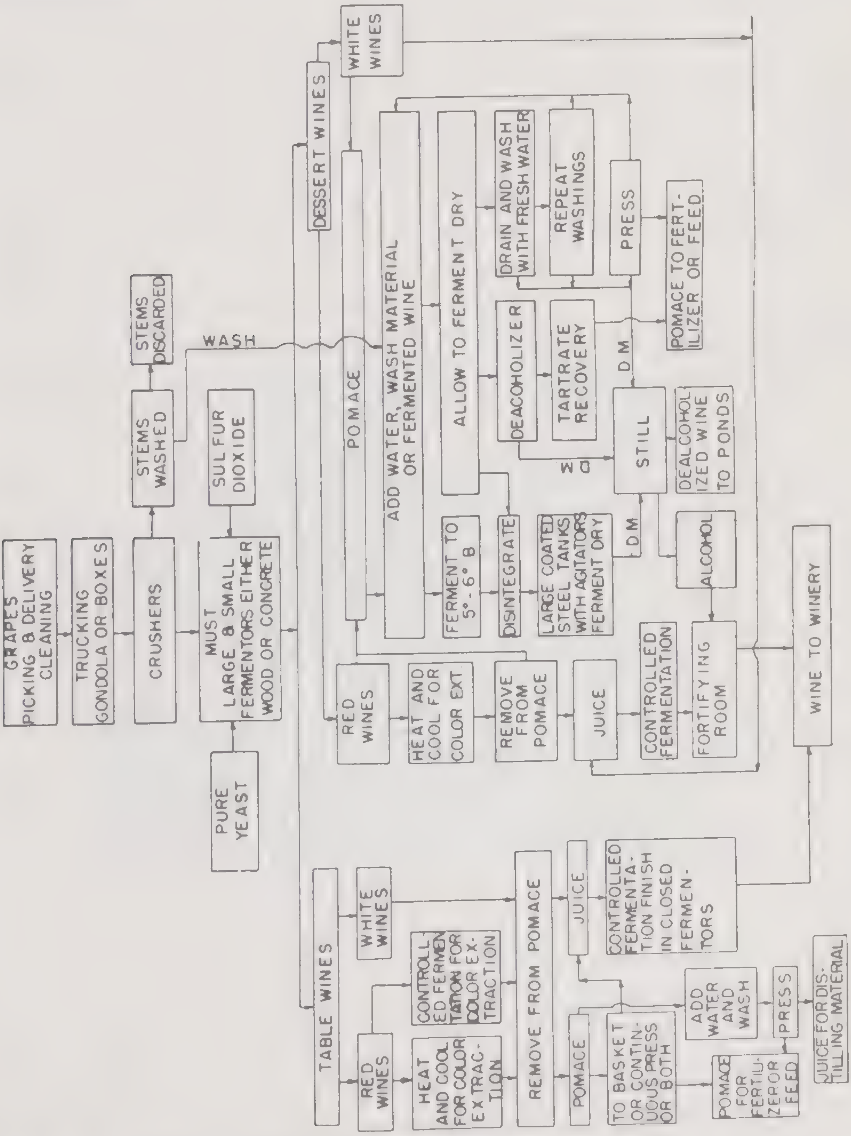


FIGURE 45. *Flow Diagram of Wine Manufacture*

## Conveyors

Conveyors are widely used in unloading and handling grapes at the winery. Modern conveyors are constructed to serve a dual unloading purpose. One side of the conveyor is built to accommodate automatic unloading and the other side to unload grapes brought to the winery in field boxes.

The side for boxes must be higher, at least as high as the truck bed. This height may vary, depending on the size of the truck, but is ordinarily between 48 and 52 in. The other side of the conveyor is much lower for automatic unloading; it should not be over 36 in. high, and 28 to 30 in. is preferable. Conveyors built of concrete throughout are not recommended because, once a concrete conveyor is poured, it cannot be moved. Wood conveyors are satisfactory, except that they require more maintenance.

The latest development in conveyor construction is to build in sections and to use steel throughout. Steel conveyors are as strong as concrete and are movable. The ordinary conveyor is 50 to 60 ft long, with an incline of from 15 to 20 ft. All conveyors should be lined on the bottom. A glass lining, which extends the life of cleats materially, has proved very satisfactory. Cleats, which are made of wood, will wear out in one season and must be replaced if they run on a wood surface. Wooden cleats running on a glass surface have lasted 3 years without showing a great deal of wear. It is thought that tile lining will prove just as satisfactory. Steel lining has given as good service as glass, but is undesirable because of metal pick-up; however, proper maintenance with acid-resisting paints will largely eliminate the metal pick-up.

The conveyor chain used depends largely on the type of operation. For an all-purpose conveyor, the No. 988 manganese bronze chain with cleat spacing of about 15 links or approximately 18 in. should be used. This chain is regulated to operate at a rate of about 60 ft per minute.

## Crushers and Stemmers

The grapes are conveyed to the crushers where the berries are crushed or macerated to liberate the juice and the stems are separated from the resulting must. Care should be taken not to macerate the skins excessively. The seeds should not be crushed, nor should the juice be expressed from the stems. The grapes may be crushed between two grooved metal rollers which crush and

tear the grapes while revolving in opposite directions at the same or at different speeds. The rollers must be set far enough apart so that the seeds are not broken, but close enough to crush all the fruit. The proper distance between them depends on the variety, maturity, and conditions of growth of the grapes. The crushed grapes and stems drop down into one end of a perforated cylinder where they are separated on rotating screens or by revolving curved blade paddles. The larger crusher-stemmers use finger-type pickers to separate the stems. Other crushers have very rapidly revolving paddles set in a more slowly revolving perforated cylinder (Figure 46). The grapes are knocked against the sides of the cylinder and crushed; the must drops through the holes in the cylinder and the stems continue to move out through the end. The yield of stems per ton of grapes will vary from 44 to 90 lb, usually 60 to 70 lb, occupying a volume of 2 to 3 cu ft. Crushers vary in size from as low as 10 tons per hour to as large as 100 tons per hour.

FIGURE 46. *Inside View of a Crusher-Stemmer*

## Must Handling

The must is pumped from the bottom of the crusher through a must line to the fermenting tanks. The piston and ball type must pumps formerly used proved to be expensive in upkeep and not entirely satisfactory. The centrifugal pump being employed almost

exclusively today has a paddle-type open runner with two vane-type blades. If the crushers and pumps are washed out every night and not allowed to cake or clog, no trouble will be encountered with the pump.

For a 50-ton per hour load, at least a 6-in. pump should be used. The gallonage handled will vary somewhat with the type of grapes being crushed. The average industrial yield of must is between 220 and 240 gal per ton of grapes. This amounts to approximately 10,000 to 12,500 gal of must per hour to be handled by the must pump. At least 5 in. lines should be used with large-radius ells and as few turns and bends as feasible; they may be of iron, copper, wood, or stainless steel construction. If pipe resistance is kept down, the flow of must is easier. The valves used in must lines should be quick acting.

The lines carrying the must from the main lines to the fermenting tanks vary with the plant (Figures 47 and 48). Modern practice is to use reinforced rubber hose. These hoses are clamped to the main lines at the valves and can be easily moved to change the flow from one fermentor to another. One man can handle 25

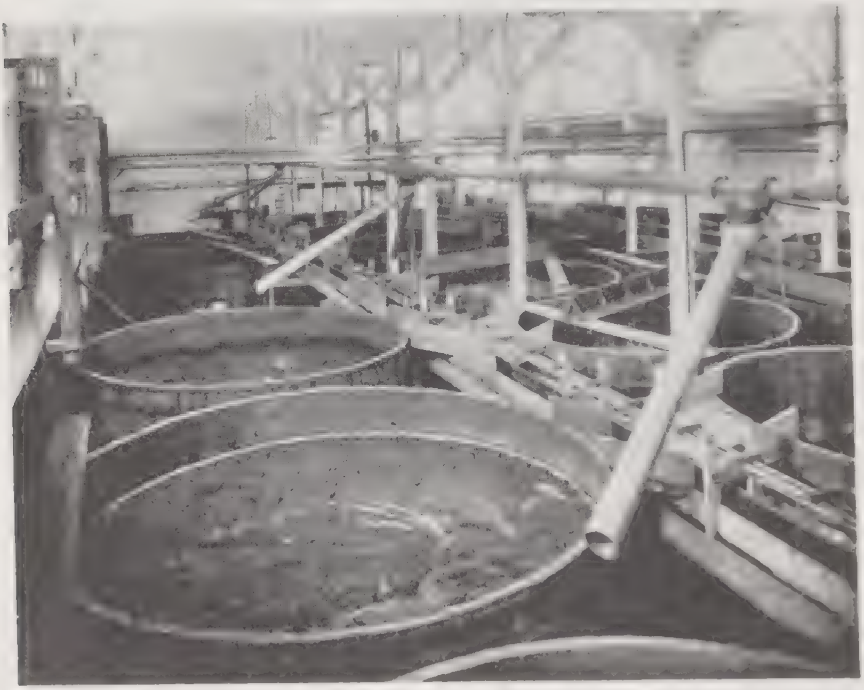

FIGURE 47. *Wine Fermentation Room*

ft of a 4-in. rubber must line without difficulty. Thus, if the must lines are run down the center of the fermenting room, overhead, with hose-line take-offs, each valve is quite capable of handling a minimum of eight fermentors. If the fermenting room has two banks of double tanks, the must line may be installed in a large "U" with take-offs at the most convenient places.

FIGURE 48. *Fermentation-Room Operations*

## Fermentation Capacity

The size and kind of cooperage or vats for the proper fermenting room will depend largely on the over-all size of the winery and the type of wines produced. On the assumption that 4 tons of crushed grapes require 1,000 gal of space, the following formula will give the space needed in the fermenting room:

$$\text{Space} = \frac{100 \times DT \times FS}{4 \times TO}$$

$DT$ = the daily grape tonnage to be received; $FS$ = the total fermenting season in days; $TO$ = the turnover, or the days required to empty a fermentor.

Thus a dry wine winery, with a 7-day turnover, a crush of 100

tons per day, and a season of 50 days, accordingly will require 175,000 gal total capacity. This allows for a 5,000 ton over-all crush. If one desires to crush more tons per day or shorten the total season, the space required will vary accordingly.

For the manufacture of good-quality dry wines, the tanks should be under 5,000 gal in size, unless ample refrigeration is provided to compensate for the lower heat losses in the larger tanks. A suggested breakdown for such a fermenting room would be six 10,000-gal, sixteen 5,000-gal, and twelve 3,000-gal size tanks. The smaller tanks should be of redwood and a large percentage (at least 25%) should be closed. The larger tanks may be of concrete, but it is advisable to have them coated when new. Microcrystalline wax has been found to be satisfactory for coating cement fermentors.

The fermenting operations will be somewhat different for a winery making dessert wine, depending on the method of handling pomace. Assuming on the average a 5-day turnover and a crush of 500 tons per day, a fermenting capacity of 1,250,000 gal will be required. For a dessert winery, concrete cooperage will prove more adequate; it is more easily cleaned, sterilized, emptied, etc. The size of tanks again would depend on the type of operation, but a suggested breakdown would be twelve 50,000-gal, ten 30-000-gal, ten 20,000-gal, ten 10,000-gal, and ten 5,000-gal capacity tanks, or some variation of this. The small tanks are desirable to properly segregate varietal types of grapes.

## Sulfur Dioxide

The use of sulfur dioxide for fermentation control should be universal. The amount added may vary from 100 to 150 ppm. Liquid sulfur dioxide in cylinders is used today. The dispensing companies furnish small cylinders containing 12 to 23 lb of liquid sulfur dioxide, equipped with glass gages graduated in pounds, so that exact quantities can be measured. These small cylinders can be refilled mechanically from large 150-lb cylinders. One pound of liquid sulfur dioxide per 1,000 gal of wine or must is equivalent to the addition of 120 ppm.

## Pure Yeast

Pure-yeast cultures should be used in all fermentations whether they are for distilling material or for wine production. Three varieties of yeasts or mixtures of them are in general use, the

Champagne, Burgundy, and Tokay yeasts. Usually a pure culture purchased from a commercial laboratory is grown in sterile grape juice until brought to a commercial quantity, then transferred to the fermenting room, and propagated throughout the season. One method is to use up at least half of the culture tank each day and to add fresh sulfited juice to the remaining half, thus keeping a vigorously growing culture on hand at all times. Close laboratory check and control of the culture is needed with this method of propagation. Another method is to use two tanks equipped with coils. One tank is used for pasteurizing and cooling fresh juice, which is then pumped to the second or yeast tank. Thus sterile juice is always added to the yeast. Many variations of these two methods are in use with essentially the same results.

## Balancing

The best time to balance wines is in the fermenting room. Generally, the addition of 2 lb of citric acid and 0.5 lb of tannin per 1,000 gal of fermenting must is used. The amounts may vary a little, depending on the wine maker. However, this quantity will set the color extracted by heating red juice. Analysis of the juice obtained from the must will usually determine the best quantities to add.

## Cooling

Proper control of temperature is essential in the fermenting room. The temperature of fermentation determines the yield and quality of wines obtained and affects all phases of wine production. The fermentation temperature should be kept under 80°F at all times and for excellent quality wines should not go over 70°F. Distilling material should not go over 85°F. As previously mentioned, cooling may be by means of cooling coils in the fermentors or by passing the juice from the fermentors through heat interchangers, with well water, cooling-tower water, or water cooled by a refrigerating agent as the cooling medium. The ideal method is, of course, automatically controlled cooling by means of direct expansion of Freon in coils in the fermentors. The initial cost of such an installation will naturally be very high. However, this method may prove best to achieve top quality.

## Records

Records are of great importance in the manufacture of good, sound wines. The following is a list of the data which should be recorded: (1) Balling of every load of grapes received and the weight and type of grapes; (2) Balling and temperature of each fermentor taken every morning and night; (3) a record of the daily work sheets. A suggested form for the fermenting room would carry the information indicated in Table 29.

TABLE 29. WINE-FERMENTING ROOM FORM

| Date | Tank No. | Type grapes | Days in fermentor | Type wine | Balling | Temp. °F | Alcohol % | Operation |
|---|---|---|---|---|---|---|---|---|
| 9/2/48 | 15 | Carignane | 2 | Dry Red | 12 | 78 | —— | Circulate and cool |
| " | 18 | Alicante | 5 | D.M.[a] | 0 | 82 | 4.3 | Drain to D.M. and water |
| " | 20 | Mataro | 0 | Port wine | 22 | 72 | | Heat and circulate |

[a] Distilling material.

This type of record allows the wine maker to follow daily each tank and each variety of grape through the fermentors and thus to have a complete record of the operations. If other data are wanted, they can be added to the form.

Data which would be pertinent but difficult to obtain during the rush of a crushing season may be listed as follows: (1) gallons of must per ton for different varieties of grapes; (2) gallons of free-run juice per ton for the different varieties of grapes; (3) gallons of water used for distilling-material production; (4) pounds of pomace obtained per ton for the various varieties. This information could be gathered at small plants where the crush is not too great, but where large tonnage is handled it is not easy to keep such accurate and complete records.

## Must or Juice Handling

For top-quality, white table wines, some yield must be sacrificed. The juice should be drawn as soon as possible from the pomace. When drawn in the first 4 to 8 hours, after crushing, a yield of 120 to 160 gal of juice per ton can be expected, depending on the types of grapes. Where dessert wines are also made, the remaining juice (sugars) in the pomace is converted into distilling material. The pomace from other wines is usually pressed for the recovery of additional gallonage. The recovery from this source depends largely

on the type and extent of the pressing. An average yield is between 20 and 40 gal. The wine produced from the recovered juice is not of as high quality as the wine produced from the initial free-run juice. It is recommended to produce white wines from the free-run juice and to convert the balance into distilling material. The finest white wine is made from free-run juice. A light press fraction may be used for a somewhat lower quality wine. Basket or continuous screw presses are used, depending on the size of operation.

For dessert wine only, the operations are somewhat simpler. The juice is drained and sulfur dioxide added. Yeast is then added to start the fermentation. When the desired Balling degree is reached, the wine is fortified. The pomace is used for the production of distilling material.

The must from red grapes is handled similarly, except for the additional step of extracting the color. This can be done in two ways: (1) heating the juice through external heat interchangers and passing it over the must; or (2) allowing the juice to ferment on the seeds and skins with almost continuous circulation.

When the first method is used, the juice should not be heated over 110°F if it is circulated over the seeds and skins for 8 to 16 hours. If the time is much shorter, higher temperatures can be used and for a longer time, lower temperatures. The mechanism of the operation also varies as to locale and type of grapes. In the north-coast counties, short periods of time and low temperatures will suffice to produce wines of good quality. In the hot interior valleys, a longer period of contact time is not detrimental. This method gives some control of the amount of astringency retained in the wine. Many Ports made in the north-coast counties are far too astringent and rough, whereas many Ports of the interior valleys are too light in color and body to be considered fine wines. The second method of fermenting on the seeds and skins frequently produces dry wines which are too astringent and Ports much too light. Generally, some sort of a balance between the two operations is recommended, i.e., to allow some of the wines to ferment in contact with the seeds and skins and the use of heat for color extraction for some of the wines. Blends of these can then be made to perfect either or both.

To obtain free-run juice from the must, all fermenting rooms should be equipped with stainless-steel screens, usually 36 in. wide by 10 ft long, with 3/32 in. perforations and designed for automatic

self-cleaning. These screens must be large enough for their purpose. Cleats and brushes, operated by motor, remove solids as they pass over the screen. The screens are placed over sumps equipped with automatically controlled pumps, their size depending upon the volume of wine to be pumped (Figure 49). Each winery represents a specific problem and requires different pumps, depending on the gallonage, the distance to be pumped, and the lift. Under average conditions, a 5 hp, 2-in. pump will deliver 2,500 to 4,000 gal per hour.

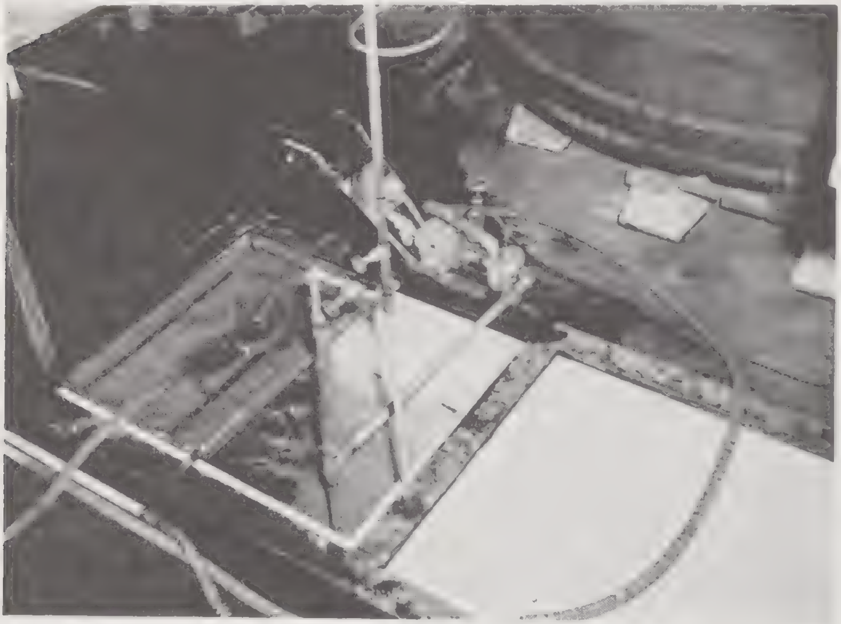

FIGURE 49. *Sumps and Sump Pumps*

The drained juice is carried to the screens either by hose lines (2-in., six-ply hose), or by gutters, depending on the extent of the operations. Gutters are usually run two on each side of the passageway between the fermenting tanks. One is used for wine and the other for distilling material. Wash-out gutters are separate. The draining gutters or hoses are run directly to the automatically controlled screens over the sumps, so that seeds and skins can be separated from the juice. Small conveyors are installed to carry the seeds and skins removed by the screens. Gutters should be covered and cleaned and sterilized daily. Hoses should be cleaned and steamed after each use.

**Pomace Handling**

The treatment of pomace depends on the type of winery operation and may be subdivided into the following categories: (1) dry white wine, (2) all dry wines, (3) dry and sweet wines, (4) all sweet wines. In the production of dry white wines, the pomace is pressed immediately after the free-run juice is drained off. It is then shoveled to basket presses or continuous presses, pressed out, and the spent pomace discarded (Figure 50). A ton of grapes yields 250 to 400 lb of pomace. The continuous presses will handle 10,000 to 15,000 lb of pomace per hour at a moisture content of 50 to 60%. With two men shoveling and one man watching, a continuous press will easily handle 800 gal of pomace per hour, which is the approximate volume of pomace obtained from 10 tons of grapes. The presses are quite capable of handling almost five times this volume.

In the production of dry wines only, the pomace would be handled as it is for all white wines, but for dry and sweet wines, there are several variations.

FIGURE 50. *Grape-Pomace Pile* (Courtesy—Wine Institute, San Francisco, Calif.)

The free-run juice may be drained and an equivalent amount of water added, i.e., to 1,000 gal of pomace in the fermentor add 1,000 gal of water. In this method, the distilling material is usually 4° Balling below that of the juice as it was drained. For example, if 20° juice is drained and water added, the Balling of the resulting juice will be 16°. Yeast is added and the mixture is allowed to ferment dry. It is then drained, and half (500 gal) the quantity of water added for a second washing. Again it is drained and one-fourth the quantity of water added. The percentage of alcohol in these last washings should be determined. When the alcohol drops below 2%, these washings are then used for a first or second washing of fresh pomace. By properly controlling this system, much water can be saved. When the alcohol of the wash drops to around 0.5% the pomace is shoveled out and the press wine is used as a first or second wash on unwashed pomace.

A variation of this system is to pump the pomace out of the tank instead of shoveling. In this case, the washings are the same, but instead of draining the last washing, a sump pump is lowered into the fermentor and the whole mass pumped to the press. In this case, the press should have a dewatering screen, approximately 20 ft long and 12 in. wide installed ahead of the press, so that the material being pumped will be dewatered before entering the press. This system will save materially on labor. Assuming that a fermentor requires two men for 4 hours to shovel out, or a total of 8 man hours, a pump can empty this same fermentor in ½ hour, requiring one man or ½ man hour plus the power for a 15 hp sump pump.

The use of pumps was introduced only within the past few years; prior to this, many wineries installed the sluice-out method. The bottoms of the fermentors were constructed with at least 6-in. slope, with a manhole at the lowest level, so that the door could be opened and the material washed out with water or low-alcohol distilling material. Both the shoveling and the sluice-out methods require conveyors to transport the pomace from the tanks to the presses. The use of pumps can eliminate these conveyors.

If a pomace still is used, the procedure again varies. After draining the free-run juice, water is added to the seeds and skins. The amount of water added depends on the alcohol desired in the resulting distilling material. The mass of seeds, skins, and water is then pumped or sluiced out to a disintegrator. The disintegrators

will be of 25 to 40 hp, depending on the type and size. This machine disintegrates the whole mass and it can then be readily pumped and distilled in a pomace still. Unless the material is kept under constant agitation, its suspended solids will tend to settle out and plug the stills.

It is unnecessary to disintegrate pomace if a Metzner type still is used (Figure 51). After the free-run juice is drained, water and yeast are added to the seeds and skins and the mass allowed to ferment dry. The first distilling material can be drained if desired

FIGURE 51. *Metzner Distilling Unit for Recovery of Alcohol from Pomace* (Courtesy—Petri Wine Co., Tulare, and Wine Publications, San Francisco, Calif.)

and water added. The pomace is then sluiced, pumped, or shoveled to the still for steam stripping.

## CELLAR OPERATIONS

The initial fermentation of wine is conducted in the fermenting room (Figure 47). The partially fermented wine is then transferred to the storage cellar for completion of fermentation, clarification, aging, stabilization, and other preparation for marketing. These

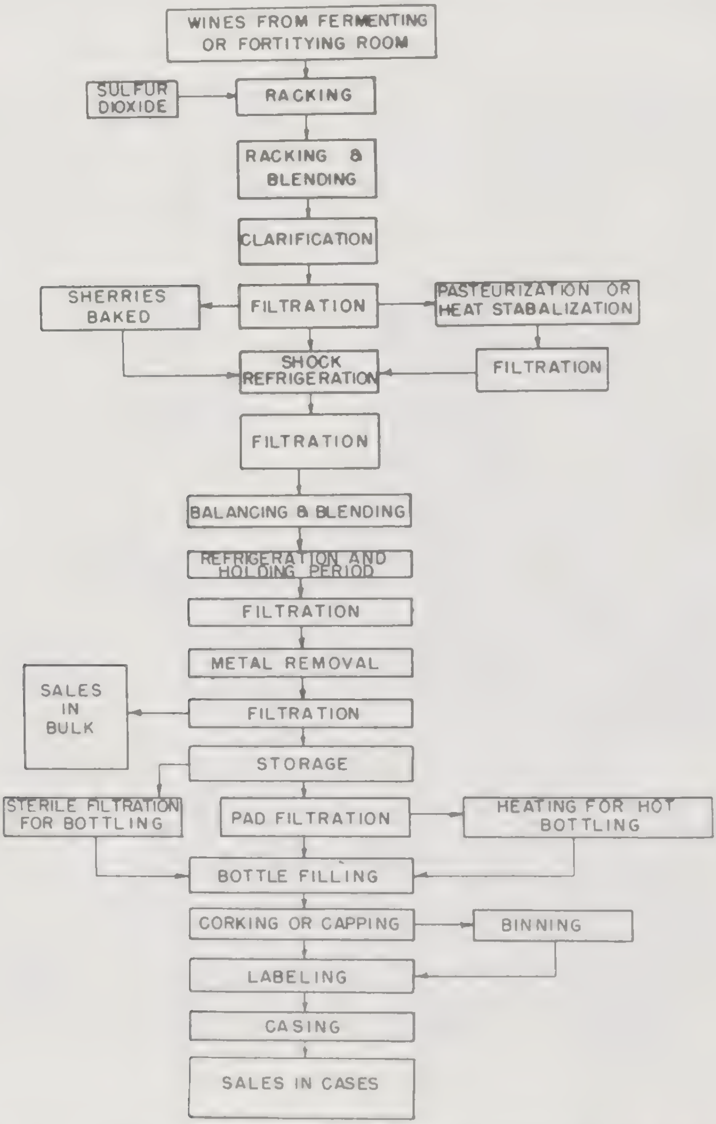


FIGURE 52. *Flow Diagram of Cellar Operations in a Winery*

operations are usually referred to as cellar practices. Typical cellar operations in a winery are summarized in the flow diagram of Figure 52.

For the proper development of dry table wines, the fermentation is not allowed to cease until all fermentable sugar has disappeared and prompt and complete attenuation obtained. After fermentation ceases, the yeast and suspended particles of pulp and skins settle rapidly and form a sediment known as the first or crude lees. When fermentation is complete the new wine is drawn off (racked) from the lees, to aid in clearing and to avoid extraction of undesirable flavors from the yeast and other solids. The cleared wine is stored in completely filled and sealed tanks for aging. Filtration, fining, and refrigeration are used in preparing the wine for the market. Fortification with grape spirits and special heat treatments are additional operations for dessert wines.

The stabilization practices used in California for the preparation of wine for market are based on the application of racking, fining, filtration, refrigeration, pasteurization, sulfiting, blending, and acidification operations in sequence and type to suit the particular needs of the wine and winery. A survey made by Berg[6] of the stabilization procedures in use in California wineries, largely developed individually by trial and error method, indicated not only a wide difference in the equipment and methods, but also in the order of application and in the number of times that a particular operation is repeated. There is no rational justification for the existing variations; there is little to justify the sequence of operations at a particular winery; and the effect of these operations on organoleptic quality is largely unknown.

Table wines are racked from the closed fermentors directly to storage tanks after fermentation is completed. Dessert wines, however, first have their fermentation arrested by the addition of brandy. After the addition of the brandy, the wines should rest for a period of 24 hours to allow for their complete settling before being transferred to the storage cellars.

Cellars should be so constructed as to maintain an almost constant temperature throughout the year. Tanks may be constructed of concrete, or redwood, or oak. The type and size of the vats will depend, to a large extent, on the method of operation. It is well, however, to have all types of vats in the storage cellars and to calculate the size so that sufficient small cooperage is available

for breakdown of larger amounts. The gallonage of the tanks should be progressively decreased, i.e., if the larger tanks are 50 thousand, then the smaller tanks can follow as 30, 20, 10, 5, 2.5, 1 thousand and a few 500 gal size. It is not good practice to mix a lot of odd-size tanks in the cellars. If the large tanks are 60 thousand, then the progression can follow as above or follow one of 36, 18, 12, 6, 3, 1.5 thousand, etc. Under any circumstances, it is best to follow one pattern to make future operations easier.

## Racking

Wines should be allowed to settle for 1 to 2 months in the storage vats. During this period, all wines should be completely analyzed and if their sulfur dioxide content is found to be low, it should be brought up to 100 to 150 ppm. The wines are then racked off their lees the first time. Certain wines, particularly those of high acidity, are sometimes left on the lees in order to promote the malolactic fermentation. In such cases, the cellars are kept warm until the desired degree of deacidification has been achieved. The slight carbonation noted in some red wines early in the spring as the cellar temperature starts to rise may be due to deacidifying organisms rather than to fermentation of residual sugar by yeast. Since their activity results in decrease in acidity, such bacterial fermentations are not desirable under California conditions. Racking and the addition of sulfur dioxide in moderate amounts are usually adequate for control.

Overoxidation during racking should be avoided, particularly with white wines which mature best in the absence of excessive aeration. These wines are best when handled under a blanket of carbon dioxide gas and when racked into vats in which the air has been replaced with carbon dioxide. During racking, care should be taken to draw off the wine from the bottom just above the crude lees and to transfer it into another storage vat via the bottom. The pumps used for wine transfer should be so selected and operated as to avoid aeration. In racking, care should be exercised not to agitate the wines excessively. It is recommended to do all pumping through side valves or even bottom valves. If, however, hose lines are used, they should be introduced over the top of the tanks and the discharge end should be lowered into the receiving tank, so that it will be submerged in order to avoid aeration of the wine. Care should also be exercised that the pumps used do not

whip excess air into the wines. Even with great care, the wines will pick up enough air for their proper maturation. Lees should be accumulated in separate tanks and, if properly cared for, additional wine can be racked off the lees from time to time.

After the first racking, the wines should be given 1 to 2 additional months to settle. Some wines will clarify themselves much more readily than others and it is not unusual to find some wines relatively clear after the second racking. In making fine wines, it is best to rack them two or three times before spring rather than to use clarifying agents. However, some wine-makers believe that one racking and a clarification will accomplish the same result without detrimental effects to the wine. Enologists are in general agreement that the wines should be racked at least once before clarifying. If possible, initial blends should be made before clarification. If after three rackings, wines do not clear themselves, clarifying agents should be used. It is generally agreed that clear wines will age better than those which are not clear. Just before the clarifying agent is added, at the same time, or immediately afterward, any tannin that the wine may require should also be added.

## Fining

Although a sound wine often becomes brilliantly clear by natural settling, cloudiness may persist. In that case, clarification can be aided best by fining, filtration, refrigeration, centrifuging, or heating. Fining hastens aging, defecation, and bottle maturity. Even the best wines are nearly always fined at least once before being bottled. In fining, the colloidal particles coalesce and coagulate. The coagulum settles by gravity, carrying with it other suspended matter that may be present.

The amount and type of fining agents to use will depend on the nature of the suspended matter and on the type of wine. The fining agents commonly used are gelatin, isinglass, casein, and bentonite. These, either by combining chemically with the colloids or by neutralizing the electrical charge of the particles, cause coagulation and settling.

The fining agent most widely used is bentonite. It is easy to handle and gives good clarification if properly employed. In the past, the general practice was to set up test clarifications in the laboratory and then use the amount necessary to give a clear wine. This amount was sometimes as high as 12 lb of bentonite per 1,000

gal of wine. These large quantities did give good clarifications but also resulted in excessive amounts of lees. In recent years, many winemakers have used quantities only large enough to form a precipitate and have followed this with a filtration. In the first method, the required amount of bentonite is added to the wine, first mixing small quantities of bentonite with small portions of the wine, then gradually adding them to the main portion, after which the whole mass is allowed to settle for approximately 10 days. During this period the wine clears and the lees settle out. The wine is then racked off the lees and filtered. In the second method, much smaller quantities of bentonite, 1 to 2 lb per 1,000 gal of wine, are used; in no case should more than 2 lb of bentonite be used per 1,000 gal of wine. The whole is properly mixed and then filtered on coalescing. Here care should also be exercised that too much air is not whipped into the wine. In this method very little lees are accumulated.

Gelatin and similar fining agents that combine with the tannin decrease the tannin content of wine and cause noticeable decrease of color. In certain light wines, where loss of color is not desirable, isinglass should be substituted for gelatin or casein. Prior addition of tannin will also prevent the bleaching of color by casein or gelatin and increase the rate of sedimentation. The fining agents dissolved in water are thoroughly mixed with the wine, which is then stored until the suspended matter settles out. It can then be racked and, if necessary, filtered.

Casein used as clarifier can be filtered soon after its addition, if the precipitate formed is made up of large particles. The use of gelatin and isinglass is a little more difficult. Solutions of these agents must be prepared beforehand and the clarification of the wine must be carried out under exacting conditions. The wines are allowed to settle for a period of 10 to 14 days, then racked and filtered. Many cases are known where difficulty has been encountered with these agents and the addition of more agent or another agent has been necessary to complete the clarification. When properly used, gelatin will give a good clarification with small quantities of lees. In all cases, the clarification is followed by a coarse filtration.

## Filtration

Filtration is usually a necessary supplement to clarification. Very cloudy wines may be filtered to clear them rapidly; bulk

filtration of this type is used also in preparing ordinary wine for the market. All wines to be bottled are given a finishing or polishing filtration just before bottling. Close filtration is used rather extensively abroad for sterilizing the wines by removing all microorganisms. Particularly in a finishing filtration, the wine must be protected from excessive aeration and from contamination with metallic impurities from the filter lines and the filter medium. The introduction of iron, copper, or calcium from poorly manufactured filter pads, filter aid, or filter mass will make the wine unstable.

The filtration is carried out in either a plate and frame filter or a screen-type filter. Most wineries now use the plate and frame filter. The filter aid (diatomaceous earth) used in this filtration is usually of a type yielding high flow rates and a product of moderate brilliance.

In the plate and frame filter press, the wine is forced through the filter medium by means of a suitable nonaerating pump. The wine is forced under pressure through canvas sheets, lined with filter paper and precoated with diatomaceous earth. The individual filter compartments are formed by corrosion-resistant metal frames and the canvas, with its paper liner and precoat, is supported by a metal plate. The filter-paper liner which has been recently introduced prevents particles of filter aid from going through the canvas and materially assists in cleaning the canvas and the press.

To minimize the sliming and clogging effect of the soft and amorphous particles, which tend to pack together and clog the filter pores, the wine is mixed with a filter aid. For best results, the filter aid must be uniformly mixed with the wine and held in suspension by constant agitation during filtration. The amount of filter aid necessary varies with its character and with the type of wine. An excess is to be strictly avoided, and the filtration should be so conducted that all particles of the aid are removed; otherwise the wine may take on undesirable flavors.

Sterilization Filtration

When filter pads of uniform porosity and of small-enough pore size are used, and the filtration is carefully conducted, all microorganisms may be removed from the wine. If such closely filtered wine is filled into sterile bottles under aseptic conditions and is closed with sterile corks, it will not be subject to microbial spoilage. Fessler[13] developed sterilization filtration practice for California

wines based on the use of asbestos-cellulose filter pads properly precoated with filter aid to seal the larger pores and sterilization of the filter assembly, bottling line, and bottles by treatment with quaternary ammonium germicides. The filtration is then conducted at such pressures that yeasts and bacteria which could grow in the bottled wines and produce turbidity are retained on the filter pads. His method has been successfully adopted in several industrial installations and has solved the problem of reinfection of white table wines and other wines (Turbovsky[33]).

### Heat Stabilization

If wines are to be prepared for marketing before they are sufficiently aged to be heat stable, they are usually heat treated. They may be heated to 140°F for 24 to 72 hours, or they may be pasteurized, i.e., heated to 180°F for 1 minute, or subjected to lower temperatures for somewhat longer periods.

Marked improvements in pasteurizer designs have been made in the past few years. Plate-type units designed to provide heat exchange and regeneration have replaced the old-type tubular heat exchangers. After pasteurizing or heat stabilizing, it is necessary to filter the wine again to remove coagulated material. This heating speeds the separation or coagulation of the proteins and gums and produces a wine that is more stable to higher temperature.

### Chilling

Wines are refrigerated to make them more stable against precipitation of cream of tartar and clouding when exposed to lower temperatures. It is desirable to keep wine, after fermentation, cold for several weeks to throw out of solution excess salts (such as cream of tartar) and to hasten the deposition of colloids. A clear wine will mature best at an even temperature between 50° and 60°F. Chilling the wine to the freezing point is an effective means of quickly eliminating cream of tartar from new wines and is useful in preparing ordinary table wines.

After heat treatment, the wine is usually given its initial cooling by passing it through a heat interchanger cooled by ammonia or Freon. The size of the machine depends on the volume to be cooled per hour and the temperature drop desired in the wine. A 40 hp unit will cool approximately 800 gal of wine per hour from cellar temperature of 60°F to around 18°F. The wine is

passed through the unit to precipitate the tartarates and filtered immediately. This initial cooling or shock refrigeration removes the major portion of the tartrates. If wine is stored for 2 to 3 weeks at low temperatures, it is not necessary to use this shock refrigeration in finishing it.

It is at this point that most of the blending and balancing of the wine is done. At this time, the wines are again analyzed and the tannin, acid, and sulfur dioxide brought to their proper concentrations. Blends are made and put away to age. In order to bring out the best qualities of the wines, it is best to blend early in order to allow the wines to "marry" and mature properly. It is generally agreed that new blends should age at least 3 months before they become properly "married."

Wines are then stored until the final finishing is to be done. This consists of a refrigeration and holding period of at least 10 days at a temperature of 15°F for dessert wines and 22°F for table wines. Maintaining the proper temperature in the wine may be accomplished in one of several ways:

(1) *Cooling coils in storage tanks.* These coils are cooled by direct expansion with Freon or methyl chloride and are installed in the top section of the tank. The amount of cooling necessary is just sufficient to overcome the heat absorbed from outside. Using redwood tanks of 20,000 gal size, with 500 ft of 1-in. coil in each tank, a 10 hp refrigeration unit will maintain the required temperature in four tanks very well. The objection to this type of installation is mainly that (a) the tanks "sweat" continually and are, therefore, quite susceptible to molding and (b) one cannot use this type of installation for table wines as the coils may become coated with ice and break. Of course, the wine must be cooled to temperature while going into these tanks.

(2) *The use of insulated tanks.* This method is good and works well. A properly insulated tank of even 50,000 gal size will lose about 5°F in a period of 10 days. The wine is cooled to the proper temperature as it is run into the tank and allowed to rest. After 10 days, it is given its final filtration.

(3) *Cold room.* This method is the best. The wines are stored in an insulated room in which the temperature is controlled by an independent compressor. The wine is cooled to the proper temperature as it is pumped into the storage tanks, and the room kept at the desired temperature. Of course, if dessert wines have been

stored in the room and it is desired to use it for storing table wines, the thermostat control should be changed. Dessert and table wines cannot be held in the room at the same time, as the table wines will freeze.

(4) *Intermittent cooling.* If none of the methods (1) to (3) is available, a circulating cooler can be used to cool the wines every 3 or 4 days. Since low temperatures cannot thus be maintained and the wine is subject to too much agitation, this method is not satisfactory.

The final filtration of the wine may be done with an asbestos coat on the filter. This is accomplished by first precoating the filter with diatomaceous earth and then adding a precoat of asbestos while still recirculating the filter. One pound of asbestos per 25 sq ft of filtering area is sufficient. Acid-washed asbestos is recommended because there is less possibility of calcium or other metal pick-up.

## Metal Removal

The presence of excessive amounts of metallic impurities (chiefly iron and copper salts) or overoxidation may produce various types of hazes or sediments. White wines containing excess iron salts will develop a colloidal ferric phosphate haze or sediment, and red wines, a ferric tannate haze. The white ferric phosphate haze will occur only in the range of pH 2.9 to 3.6, and can be controlled usually by addition of 1 or 2 lb of citric acid per 1,000 gal of wine. In sulfited white wines containing over 0.2 ppm of copper, stored in sealed containers, a reddish-brown deposit of colloidal cuprous sulfide or a white haze will form. The excess of copper and iron can be removed from the wine by treatment with potassium ferrocyanide under controlled conditions. In several European countries, this so-called blue-fining is permitted when practiced under government supervision. Blue-fining is usually accompanied or followed by fining with gelatin-tannin, casein, or bentonite.

Several possible substitutes for soluble ferrocyanides have been proposed in recent years (Joslyn, Cane, and Lukton[21]), but of the treatments investigated, an adsorption complex of Prussian blue and ferrocyanide, or a new ferrocyanide preparation developed by Fessler[13] is the most promising. The Fessler compound has been found to remove excess copper from wine effectively and can be added in larger amounts than required to remove the copper with-

out change in chemical composition or alteration in organoleptic qualities. Its use was permitted in 1952 in California by the California State Department of Health under control of a qualified food technologist (Marsh[25]).

### Control of Bacterial Spoilage

The most serious spoilage in musts and wines is caused by the unchecked activity of acetic and lactic acid bacteria. The spoilage of must can be readily checked by the use of sulfur dioxide, cool fermentation and pure yeast. The most widely distributed acid-tolerant organisms responsible for the spoilage of wine are gram-positive, lactic-acid-producing bacteria of both rod and spherical forms. *Leuconostoc* forms are more prevalent than *Lactobacillus* species. *Lactobacillus* spoilage of table wines can be checked by pasteurization (about 1 minute at 145°F) and maintenance of a sulfur dioxide content of about 100 ppm or by sterile filtration. The most common and serious bacterial spoilage of fortified wine is caused by an alcohol-tolerant, filamentous, heterofermentative species of *Lactobacillus*. Sulfur dioxide pasteurization or acidification may be used to control spoilage by this organism.[12,14]

Yeast turbidities have been observed in white table wines and in certain dessert wines, particularly those of low free sulfur dioxide content. Sterilization-filtration has been found the best means of controlling these conditions.

## SPECIAL CELLAR PRACTICES IN DESSERT-WINE PRODUCTION

### Fortification

In the production of dessert wines, sound, clean brandy, preferably obtained from the varieties of grapes used in making the particular wine, is added to the fermented or partly fermented wine under the jurisdiction of a government gager. The wine to be fortified is pumped from the fermentor at a Balling reading 6° to 10° higher than is desired at the time of fortification. The fortification is carried out in a special "fortifying room," which is equipped with closed fortifying tanks of the necessary capacity, closed storage tanks for brandy, and brandy-weighing tank. The wine to be fortified is thoroughly mixed, usually with clean compressed air,

its alcohol content determined by the gager, and sufficient brandy added and mixed with the wine to bring this to about 21%.

Occasional lots of wine are fortified to as low as 19% or as high as 24% for blending purposes. The standard wines are marketed at about 20% alcohol, California Port testing 6° Balling, Muscatel and Angelica 7° Balling or over, and Sherry —3° to 3° Balling. Due to the effect of added brandy, the Balling degree, after fortification, will be 6° to 10° lower than before fortification.

### Heat Treatment

In addition to the usual heat stabilization, California Sherry wines receive a special heat treatment or baking to develop the desired flavor. The sherry material is prepared for this by storage, aging, clarification, and filtration. In the first years after repeal of prohibition, sherries were baked without any prior treatment; the break-point was the criterion for determining when the sherry was done, i.e., when a sample withdrawn from the tank and allowed to cool would coagulate and clear itself within 24 hours. If the sample remained cloudy, the sherry needed additional baking. However, it is now believed that baking a clean wine will produce a finer sherry, the amount of baking necessary being determined by the taste of the individual wine maker.

The most common method of heating is the use of live steam through closed coils in the bottom of the tank. Another is to pass hot water through coils. A third method gaining favor is to heat the wine with an external heat interchanger and to store the wine in insulated tanks or tanks in an insulated room. The wine is brought up to temperature at regular intervals. Heating this wine stabilizes it as well.

### Vermouth

Vermouth is essentially a basic fortified wine with an infusion of a characteristic mixture of bitter and aromatic herbs. Two types of vermouth are recognized, the Italian or Sweet Vermouth of 15 to 17% alcohol and 12 to 19% reducing sugar, and the French or Dry Vermouth of about 18% alcohol and 4% reducing sugar. The wine base used in making American vermouths is aged, clarified and stabilized. The desired extractive from the herbs and drugs may be obtained by suspending the ground or macerated herbs directly in the wine base or by preparing a water or alcohol extract which is

then added as a flavoring agent. The newly made vermouth should be fined, aged in wood for a short period of time, and then bottled.

## SPECIAL PRACTICES IN TABLE-WINE PRODUCTION

### Sweet Table Wines

In the production of sweet table wines, such as the Sauternes, musts with higher sugar content are used and the fermentation is checked while considerable quantities of sugar are still present. This is best done by controlling the rate, extent, and character of fermentation by cooling and by frequent careful rackings, followed by addition of small doses of sulfur dioxide. Sweet table wines may also be produced by blending the dry table wines with grape concentrate prepared from sulfited must. The last procedure is used in the preparation of sweet clarets and similar wines.

### Sparkling Wines

Various types of wines retaining a permanent excess of carbon dioxide are produced. These are distinguished from one another by their method of manufacture or by the base wine used. The largest volume of sparkling wine, which may be labeled "Champagne," is a white variety produced by a secondary fermentation in a closed container of 1-gal capacity or less. Wines produced by a secondary fermentation in large-sized closed containers (Charmat and other processes) are called champagne-type or "Champagne-bulk process" if they have the aroma, taste, and characteristics generally attributed to Champagne. Sparkling Burgundy is a red wine produced by a secondary fermentation in a closed container. Various sparkling pink wines are also made. In addition, carbonated wines of various types are produced, which are artificially charged with carbon dioxide. For all purposes, a wine low in volatile acidity, tart, brilliant, and clean is required.

In the production of "Champagne," a wine base prepared from appropriate varieties (Chardonnay, Pinot blanc, and Pinot noir in California, and selected varieties of eastern grapes in New York) is used. The base wine must be below 0.07% in volatile acidity, between 11.5 and 12.5% in alcohol, and above 0.70% in total acidity. The selected wine base is mixed with the required amount of sugar, a pure culture of the agglomerating champagne-type yeast,

citric acid and tannin if required, and then bottled in heavy-walled glass bottles capable of withstanding pressures up to 9 atm. The bottles are stoppered with special three-piece paraffined corks, called tirage corks, which are held in place by a special steel clamp, called an agrafe. The bottled wine is then allowed to ferment in a cool cellar until the desired amount of carbon dioxide (4-6 atm pressure) is produced. About 4.3 g of sugar per liter of wine will yield 1 atm of pressure in the bottle.

After about 6 months, when the fermentation is practically over, the precipitation of yeast cells, tartrates, etc., is facilitated by shaking the bottles and lowering the temperature to 25°F for 2 weeks. Clarification is then accomplished by gradually moving the sediment on the side of the bottles to the corks while they are stored on special racks, neck down. The sediment is removed from the bottles by disgorging, i.e., by freezing the sediment and a portion of the liquid in the bottle neck, removing the clamp, and allowing the plug to be forced out by the gas pressure in the bottle. The wine, after disgorging, is free of sugar and very dry in taste. To satisfy the consumer's palate, a liqueur of pure sugar, aged wine, and brandy is added and the bottle is corked, wired, capped, and stored for aging.

In the bulk-fermentation processes, it is possible to produce a large volume of uniform sparkling wine of the desired composition and at a lower cost than by the regular champagne method. The tanks are usually of steel, lined with glass or with corrosion-resistant metal, and are provided with means of cooling or heating the wine. A yeast starter is prepared with sulfite-adapted yeast in diluted grape concentrate or in wine, supplemented with ammonium phosphate, urea, or wine malt. The wine base (usually of about 11.5 to 12.0% alcohol and 0.16% sugar content) is brought to 0.75% acidity by the addition of citric acid and enough invert-sugar sirup is added to produce a cuvée of 12.5% alcohol, 2% reducing sugar, and a carbon dioxide pressure of 6 atm. In such bulk fermentation, 4 g of sucrose per l will produce 1 atmosphere pressure. The wine is brought to 100 ppm of total sulfur dioxide, about 5% of active sulfite-adapted yeast starter is added, and the fermentation is conducted at 60°F. Cold brine is circulated through the cooling jacket to maintain this temperature. The carbon dioxide pressure increases steadily during fermentation and usually reaches 90 lb at the end of 6 days. When the wine has attained this pres-

sure, the fermentation is stopped by circulating refrigerated brine through the jacket until a wine temperature of 24° to 30°F is reached. The wine is stabilized by refrigeration for 10 days at 24° to 28°F, and filtered from the fermenting tank into a precooled holding tank under isobarometric conditions. The filtered wine is then held again for about 2 days at 24°F and the cycle repeated to obtain a clear, stable wine. In some practices to stabilize the wine, sulfite is added at the end of the fermentation immediately after chilling. The wine is then bottled at below 30°F, corked, capped, and aged for 3 to 6 months.

## WINERY BY-PRODUCTS

The stems, pressed pomace, and crude lees are the main by-products of the fermentation room. Wine lees and argols are obtained as additional products in the storage cellars. To dispose of the 45 to 90 lb of stems obtained per ton of grapes crushed, they are scattered through the vineyard where they soon dry up. The pressed pomace varies from 300 lb to over 500 lb. The pomace may contain fairly large amounts of sugar or be practically free from sugar, depending on the type of operation and may be free from or contain several percent of alcohol. The pomace has little value as fertilizer but has some value as feed. The alcohol present in the pomace is recovered by distillation in a pomace still or by washing and distilling the wash. The grape seeds, which constitute 20 to 30% of the pomace on the wet basis are valuable for their oil content which ranges from 12 to 22%. The seeds may be separated from the pomace by screening, dried, and extracted with solvents to produce bland table oil.

Per ton of grapes 210 to 220 gal of crushed grapes are obtained, which yield 120 to 150 gal of free-run juice and 30 to 50 gal of press juice, giving a total of 165 to 185 gal. The free-run wine varies from 130 to 175 gal; the free-run and press wine from 160 to 176 gal. The lees from the free-run wine at first racking vary from 2 to 7 gal per ton; at the second racking, 2 to 9 gal of lees are obtained. The total volume of wine lees from two rackings varies from 4 to 13 gal. The volume of table wine obtained after two rackings is 125 to 165 gal per ton of grapes crushed. The yield of dessert wine will vary from 80 to 100 gal per ton, depending on the sugar content of grapes and wine. The lees obtained from 76,000 gal of dessert wine at the first racking amounted to 1,200 gal and at the second racking,

to 500 gal, or approximately 1.5 and 0.7% by volume, respectively. The alcohol in the lees is recovered by distillation. Both the pomace and the lees are valuable sources of cream of tartar.

## Cream of Tartar

The production of tartrates from California crudes is now limited, but during World War II, a high percentage of tartrates was recovered. Many European wineries save all their pomace, lees, and distillery slop and regularly scrape their tanks in order to recover the cream of tartar. The lower cost of production in Europe than in America allows the imported product to undersell the domestic.

The recovery of tartrates from winery wastes has been investigated by Marsh[22] who has described the principles involved in successful tartrate recovery both as cream of tartar and calcium tartrate. Matchett, Legault, Nimmo and Notter[26] have described the use of ion exchangers in acid-carbonate cycle for tartrate recovery. The extent of tartrate recovery in California from January 1942 to April 1943 has been estimated at about 2,000,000 lb of dried wine lees and argols and 900,000 lb of calcium tartrate. The winery wastes which serve as sources of tartrates are pomace, natural lees, refrigerated lees, and argols.

Table wine pomace ranges from 11 to 16% total tartrate content expressed as potassium acid tartrate for red wine pomace and 4 to 11% for white pomace on a dry weight basis. Tartrate is present in California pomace in the form of minute crystals adhering to skins and other tissue debris. It may be most economically recovered by hot-water extraction; part of the tartrates in the extracts can be recovered as crude cream of tartar by allowing the extracts to cool and the balance, by precipitation with lime and calcium chloride under controlled conditions. A pH of 4.5 should be maintained to obtain the greatest yield of calcium tartrate of highest purity. Table wine lees will contain 0.2 to 0.7 lb of potassium acid tartrate per gal and 5 to 13% alcohol. The tartrates can be recovered from the lees after the alcohol is removed by distillation. The argols, both natural and refrigerated, contain cream of tartar. The distillery slops can also be processed for tartrate recovery. In the accumulation and storage of tartrate, particular attention should be given to the control of microorganisms which may cause

decomposition (Vaughn and Marsh,[34] Stadtman, Vaughn and Marsh,[32] and Vaughn, Marsh, Stadtman, and Cantino[35]).

## EXAMINATION AND ANALYSES

Wines are regularly examined and analyzed to follow their development, detect incipient spoilage, establish type and quality, and decide on the necessary treatments and blends as well as on the time of bottling. The appearance, odor, and flavor of wine are determined by experienced wine tasters. The wine is also analyzed regularly for its alcohol, total acidity, volatile acidity, reducing sugar, tannin, and sulfur dioxide content. The composition of wines is useful as a guide to conformity to type and general soundness. Detailed data on the composition of California wines submitted for judging at several expositions has been given by Amerine.[1] Data for alcohol, extract, sugar, total acid, volatile acid, pH, iron, sulfur dioxide, tannin, color, acetaldehyde, total neutral ester, volatile neutral ester, and glycerol were shown for a large number of samples of thirteen types of white table wines, six types of red table wines, and ten types of dessert wines. Samples of the wine are subjected to various stability tests.

The Balling or Brix degree of the dessert wine is usually determined as a measure of its conformity to class designation.

(1) The clarity and color are noted on the original sample. This can be done by a Klett-Summerson or a Lumitron photoelectric colorimeter. (2) One sample is stored at 18°F and another sample at 120°F for 2 days each. (3) The readings are taken. (4) Both samples are stored at 18°F for an additional 2 days and readings taken again. (5) Both samples are stored at room temperature for 2 days and readings taken. If there is no material change in the readings or appearance of the wine, it is considered stable. Different laboratories use modifications of the described procedure and are able to forecast the stability of the wine as to heat, cold, and metallic hazes.

## PRODUCTION STATISTICS

The total apparent consumption of wine in the United States in 1947 amounted to about 97,000,000 gal, of which 86,000,000 gal were California wines. Of the balance, 8,500,000 gal were produced in other states and 2,250,000 gal were foreign wines. Of this total, 23,000,000 gal were table wines; 70,000,000 gal dessert wines;

2,300,000 gal vermouth; and 1,200,000 gal sparkling wines. The apparent consumption in 1946 was 140,500,000 gal, but included withdrawals far in excess of actual consumption. The apparent average per capita consumption was 0.67 gal in 1947 and 1.00 gal in 1946. The average for 1946-47 of 0.84 gal compares favorably with the all-time-high rate of 0.85 gal reached in 1942. The net production of wine in the United States reached 165,000,000 gal in 1946-47, of which California produced 157,000,000 gal. This quantity of wine in California was made from some 1,652,000 tons of grapes out of a total grape crop of 2,918,000 tons. The total number of bonded wineries in the United States during the years 1934 through 1948 varied between 744 and 1206, with 799 operating in 1948. The total number of California wineries varied between 381 and 733, with 381 operating in 1948.

## LITERATURE CITED

1. Amerine, M. A., *Wines and Vines,* **28**, No. 1, 21, 42, 45; No. 2, 24; No. 3, 23, 42 (1947).
2. Amerine, M. A., *Wine Rev.,* **17**, No. 1, 18; No. 2, 10, 22; No. 3, 10; No. 5, 10; No. 6, 11, 14; No. 7, 6, 19 (1949).
3. Amerine, M. A., *Wines and Vines,* **33**, No. 1, 15; No. 2, 29; No. 3, 24; No. 4, 59 (1952).
4. Amerine, M. A., and A. J. Winkler, "Grape Varieties for Wine Production," *Calif. Agr. Expt. Sta. Circ., No. 356,* 15 p. (1943).
5. Amerine, M. A., and A. J. Winkler, *Hilgardia,* **15**, No. 6, 493 (1944).
6. Berg, H. W., *Proc. Am. Soc. Enologists,* 2nd Annual Meeting, Davis, Calif., Aug., 1951, p. 90-147. Summarized by W. S. Richert, *Wines and Vines,* **33**, No. 4, 65; No. 5, 17; No. 6, 23 (1952).
7. Bioletti, F. T., *Calif. Agr. Expt. Sta. Bull., No. 174,* 27 p. (1906).
8. Bioletti, F. T., and W. V. Cruess, *Calif. Agr. Expt. Sta. Bull., No. 230,* 118 p. (1912).
9. Bouffard, A., *Progrès agricole et viticole,* **24**, 345 (1895).
10. Castelli, T., *Annali di microbiologia,* **2**, 1 (1942).
11. Cruess, W. V., *Ind. Eng. Chem.,* **4**, 581 (1912).
12. Douglas, H. C., and L. S. McClung, *Food Research,* **2**, 471 (1937)

13. Fessler, J., *Wines and Vines,* **30**, No. 6, 23 (1949); *Wine Rev.,* **17**, No. 5, 14; No. 6, 9 (1949).
14. Fornachon, J. C. M., *Bacterial Spoilage of Fortified Wines,* Australian Wine Board, 1943.
15. Genevois, L., *Ann. Fermentations,* **2**, 65 (1936).
16. Genevois, L., E. Peynaud, and J. Ribèreau-Gayon, *Compt. rend.,* **223**, 693 (1946); *Compt. rend.,* **226**, 126 (1948).
17. Haagen-Smit, A. J., F. N. Hirosawa, and T. H. Wang, *Food Research,* **14**, 472 (1949).
18. Hennig, K., and F. Villforth, *Vorratspflege u. Lebensmittelforsch.,* **5**, 181, 313 (1942).
19. Ingram, M., *J. Soc. Chem. Ind.,* **67**, 18 (1948).
20. Jacob, H. E., "Grape Growing in California," *Calif. Agr. Expt. Sta. Service Circ., No. 116,* 83 p. (1947).
21. Joslyn, M. A., A. Lukton, and A. Cane, *Food Tech.,* **7**, 20 (1953).
22. Marsh, G. L., *Proc. Inst. Food Tech.,* **1943**, 183.
23. Marsh, G. L., *Refrigerating Data Book, Refrigeration Application,* p. 277-282, New York, Am. Soc. Refrig. Engrs., 1946.
24. Marsh, G. L., *Automatic Temperature Control Recommended for Improved Wine-Growing, Calif. Agriculture,* **2**, No. 4, 5, 8 (1948).
25. Marsh, G. L., *Wines and Vines,* **33**, No. 6, 19 (1952).
26. Matchett, J. R., R. R. Legault, C. C. Nimmo, and G. K. Notter, *Ind. Eng. Chem.,* **36**, 851 (1944).
27. Moreau, L., and E. Vinet, *Rev. vit.,* **87**, 25, 61 (1937)
28. Porchet, B., *Swiss Agric. Yearbook,* p. 137-154 (1931).
29. Power, F. B., and V. K. Chestnut, *J. Am. Chem. Soc.,* **43**, 1741 (1921); *J. Agr. Research,* **23**, 47 (1923).
30. Rahn, O., *Physiology of Bacteria,* p. 27, Philadelphia, Blakiston, 1932.
31. Sale, J. W., and J. B. Wilson, *J. Agr. Research,* **33**, 301 (1926).
32. Stadtman, T. C., R. H. Vaughn, and G. L. Marsh, *J. Bact.,* **50**, 691 (1945).
33. Turbovsky, M., *Wines and Vines,* **30**, No. 6, 22 (1949).
34. Vaughn, R. H., and G. L. Marsh, *Wines and Vines,* **24**, No. 11, 26 (1943).
35. Vaughn, R. H., G. L. Marsh, T. C. Stadtman, and B. C. Cantino, *J. Bact.,* **52**, 311 (1946).
36. Willson, K. S., W. O. Walker, C. V. Mars, and W. R. Rinelli, *Fruit Products J.,* **23**, No. 3, 72 (1943).
37. Winzler, R. J., and J. P. Baumberger, *J. Cellular Comp. Physiol.,* **12**, 183 (1938).

## Additional References on Wine Making


Amerine, M. A., and M. A. Joslyn, "Commercial Production of Table Wines," *California Agr. Expt. Sta. Bul., No. 639,* 143 p. (1940).

Amerine, M. A., and M. A. Joslyn, *Table Wines. The Technology of Their Production in California,* 397 p., Berkeley, Calif., Univ. of Calif. Press, 1952.

Arrazola, Juan Marcilla, *Tratado practico de viticultura y enologia espanolas,* Vol. 1, *Viticultura,* 386 p., S.A.E.T.A., Madrid, Spain, 1942.

Arrazola, Juan Marcilla, *Tratado practico de viticultura y enologia espanolas,* Vol. 2, *Enologia,* 501 p., S.A.E.T.A., Madrid, Spain, 1946.

Babo, V., and E. Mach, *Handbuch der Kellerwirtschaft,* zweites Band, 6-te Auflage, Part 1, 560 p., 5-te Auflage, Part 2, p. 545-1088, Berlin, Paul Parey, 1922-1927.

Berg, H. W., Present Practices in California Wine Industry, *J. Agr. Food Chem.,* **1**, 152-157 (1953).

Brunet, R., and R. Chaminade, *Les vins de liqueur.* 80 p., Paris, J.-B. Baillière et fils, 1927.

Carpentieri, F., *Enologia teorico-pratica,* 12th ed. of O. Ottavi, 741 p., Casa Editrice, Casale Monferrato, Italy, F. Ottavi, 1931.

Cruess, W. V., *The principles and practice of wine making,* 2nd ed., 476 p., New York, Avi Publishing Co., 1947.

Cruess, W. V., "Investigations of the Flor Sherry Process," *California Agr. Expt. Sta. Bul. No. 710,* 40 p. (1948).

Dormontal, Charles, *Sauternes,* 213 p., Bordeaux, France, J. Biere, 1930.

Fabre, J.-Henri, *Traité encyclopédique des vins,* I. *Procédés modernes de vinification,* 4th ed., 336 p., Paris, Dujardin-Salleron, 1929.

Fabre, J.-Henri, *Procédés modernes de vinification,* II. *Analyse des vins et interprétation des résultats analytiques,* 2nd ed., 346 p., Alger, La Typo-Litho et J. Carbonel Réunies, 1936.

Garoglio, P. G., *Vinificazione razionale,* 8th ed., 254 p., Florence, Italy, "Garoglio," 1935.

Garoglio, P. G., *Trattato di enologia, enciclopedia viti-vinicola moderna,* 5 vols., Italy, Stamperia Fratelli Parenti de G. Firenze, 1941, 1942.

Genevois, L., and J. Ribèreau-Gayon, *Le vin,* 150 p., Paris, Hermann et Cie, 1947.

Goldschmidt, Fritz, *Der Wein von der Rebe bis zum Konsum,* 6th

ed., 921 p., Mainz, Germany, Verlag der Deutschen Wein-Zeitung, J. Diemer, 1925.

Hennig, K., *Chemische Untersuchungsmethoden für Weinbereiter und Süssmosthersteller*, 2nd ed., 101 p., Stuttgart, Eugen Ulmer, 1946.

Jaulmes, P., *Analyse des vins,* 574 p., Montpellier, Librairie Pontain, 1951.

Joslyn, M. A., and M. A. Amerine, "Commercial Production of Dessert Wines," *California Agr. Expt. Sta. Bul., No. 651,* 186 p. (1941).

Pacottet, P., and L. Guittonneau, *Vins de champagne et vins mousseux,* 412 p., Paris, Librairie J.-B. Baillière et fils, 1930.

Peynaud, E., *Contribution à l'étude biochemique de la maturation du raisin et de la composition des vins,* 93 p., Lille, France, G. Sautai & fils, 1948.

Ramires, A. B., *Tratado de vinifacacao,* Vol. I, 537 p., Vol. II, 446 p., Lisbon, Portugal, J. Rodrigues and Co., 1929-1931.

Ribèreau-Gayon, J., *Traité d'oenologie, transformations et traitements des vins,* 546 p., Paris, Librairie Polytechnique, Ch. Béranger, 1947.

Ribèreau-Gayon, J., and E. Peynaud, *Analyse et controle des vins,* 756 p., Paris, Librairie Polytechnique, Ch. Béranger, 1947.

Theron, D. J., and C. J. G. Niehaus, "Wine Making," *Union of South Africa Dept. of Agr. Bul. No. 191,* 98 p. (1938).

United States Tariff Commission, "Grapes, Raisins and Wines," *U. S. Tariff Comm. Rept. 2d ser. No. 134,* 408 p. (1939).

United States Treasury Dept. Bureau of Internal Revenue, *Regulations No. 7 Relative to Production, Fortification, Tax Payment, etc., of Wine,* 289 p., 1945.

Valaer, Peter, *Wines of the World,* 576 p. New York, Abelard Press, 1951.

Ventre, Jules, *Traité de vinification pratique et rationnelle,* Vol 1, *Le raisin; les vinifications,* 490 p.; Vol. 2, *Le vin, sa composition, ses maladies, sa conservation,* 487 p.; Vol. 3, *Sous-produits de la vigne et du vin,* 440 p., Montpellier, France, Librairie Coulet, 1930-1931.

Weinmann, J., and L.-F. Telle, *Manuel du travail des vins mousseux,* 335 p., Reims, France, Hirt et Cie, 1929.

Wine Institute, Annual industry statistical surveys; 12th survey published as *Bulletin No. 384 & 385,* (Mimeo.), San Francisco, Calif., Wine Institute, 1937-1948.

CHAPTER 8

# GLYCEROL

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Glycerol, also called glycerin or propanetriol, is a viscous, odorless and colorless liquid having a slightly sweet taste. It is a trihydric alcohol with a hydroxyl group on each of three adjacent carbon atoms, the formula being $CH_2OH{\cdot}CHOH{\cdot}CH_2OH$. As a chemical substance, it has many uses in such diversified products as explosives, metals, foods, beverages, cosmetics, plastics, paints, and protective coatings, to mention but a few. Leffingwell and Lesser[21] have published a rather detailed book on the industrial and commercial applications of glycerol. Included in this book is a list of 1583 individual uses for glycerol and this list is doubtless incomplete.

The principal source for glycerol has long been from the saponification of fats and oils in making soaps. It may also be produced by fermentation and by synthesis from hydrocarbon gases. Lawrie[20] in 1928 covered the literature up to that time on the subject of glycerol in practically all of its aspects.

As a product of fermentation, glycerol was first identified by Pasteur.[28] While studying pure yeast cultures in connection with the production of wines and beers, he found that 2.5 to 3.6 g of glycerol was normally produced from each 100 g of sugar fermented.

About 1911, Neuberg and coworkers began to publish results

of their investigations on the problem of the alcoholic fermentation mechanism. These studies led Neuberg to the idea of fixing the acetaldehyde formed as intermediate in the yeast fermentation by agents such as sulfites. Neuberg used sodium sulfite for most of his experiments. Since the bisulfite radical is concerned with the fixation of acetaldehyde, Neuberg suggested the following theoretical reaction:

$$C_6H_{12}O_6 + Na_2SO_3 + H_2O \longrightarrow CH_3CHO{\bullet}NaHSO_3 + NaHCO_3 + C_3H_8O_3$$

As a result of the theoretical studies, Neuberg, Hirsch, and Reinfurth[27] proposed three forms of sugar dissimilation by yeast:

I. $C_6H_{12}O_6 \longrightarrow 2\ C_2H_5OH + 2\ CO_2$ (normal alcohol fermentation)

II. $C_6H_{12}O_6 \longrightarrow CH_3CHO + CO_2 + C_3H_8O_3$ (sulfite fermentation)

III. $2\ C_6H_{12}O_6 + H_2O \longrightarrow 2\ CO_2 + CH_3COOH + C_2H_5OH + 2\ C_3H_8O_3$ (alkaline fermentation)

Neuberg's first form of fermentation, the normal alcoholic fermentation, is generally known as the Gay-Lussac reaction. His third form may be considered as merely a modification of the second form in which the alkali causes the acetaldehyde to undergo the Cannizzaro reaction to form acetic acid and ethanol.

After Neuberg's work, a great many workers have made contributions to the complete elucidation of the mechanism of yeast fermentation. The present knowledge has been outlined in Figure 1 of Chapter 2. According to this Meyerhof-Parnas-Embden scheme, the glycerol regularly found as a minor product of yeast fermentation is formed by reduction of glyceraldehyde phosphate produced during the "initial phase" of the fermentation. When enough acetaldehyde has been formed in this initial phase to serve as hydrogen acceptor, the "stationary condition" of the fermentation is reached and the normal alcohol fermentation continues. Obviously, if the acetaldehyde is fixed by addition of sulfite, or is converted to acetic acid and ethanol by alkaline conditions, it cannot serve as hydrogen acceptor, but instead, the glyceraldehyde phosphate continues to be reduced and glycerol accumulates. In this manner, therefore, the modern mechanism accounts for Neuberg's second and third forms of yeast fermentation.

During World War I, demand for glycerol for production of explosives exceeded the supply. The shortage was especially acute in Germany, cut off from importation of fats by the blockade of the Allies. The theoretical considerations of Neuberg were, therefore,

used by Connstein and Lüdecke[4] as a basis for development of fermentation procedures for glycerol production. They first investigated fermentation under alkaline conditions to obtain increased yields of glycerol. They compared various alkaline salts under varying concentrations and obtained yields of glycerol up to about 16% of the weight of sugar fermented. However, they found that alkaline conditions increased the danger of infection of the fermentation by lactic acid organisms and other contaminants which lowered yields and increased the difficulty of determining and recovering the glycerol. They, therefore, investigated the use of sulfites and developed the sulfite process for glycerol production. During World War I, after their process was developed, dynamite grade of glycerol was produced in large amounts in Germany and Austria by the yeast fermentation of a beet-sugar medium in the presence of sodium sulfite. After the war, the process was patented by Connstein and Lüdecke in many countries. United States patents[5,6] were granted in 1921 and 1924. A number of other workers in various countries later investigated fermentations for production of glycerol by addition of various soluble sulfites to yeast fermentations, for example, Cocking and Lilly,[3] Tomoda,[33] Hesse,[17] Haehn,[15] and Cornée.[7]

During World War I, information was received in the United States that Germany was producing glycerol from sugar by a fermentation process. Three government laboratories started work on the problem and Eoff, Linder, and Beyer[10] reported that the addition of alkalies, such as sodium carbonate, gave rise to considerable quantities of glycerol during the fermentation of sugars by yeast. Eoff[9] was granted a patent which covered the production of glycerol by fermentation of sugars in the presence of alkali. Large-scale test fermentations were conducted by Eoff, using blackstrap molasses as substrate. He obtained 20 to 25% of glycerol based on the fermentable sugars. Recovery of the glycerol from these fermentations proved extremely difficult. More recently McDermott,[23] Krug and McDermott,[19] Hodge,[18] and Grover[14] have patented alkaline fermentation processes, the patents of Krug and McDermott and of Hodge employing ammonia as the alkalizing agent.

May and Herrick[22] briefly reviewed the problem of producing glycerol by fermentation. More recently Whalley[37] has abstracted articles and patents through 1941, involving the production of glycerol by fermentation.

## FERMENTATION PROCESSES

The two fermentation processes of historical interest are the soluble sulfite process and the alkaline process. In the first, large amounts of soluble sulfite, such as sodium sulfite, are employed in yeast fermentations. During the fermentation, the sulfite, in contact with carbon dioxide, furnishes sodium bisulfite to fix acetaldehyde and, therefore, increases the glycerol yield. In the alkaline process, alkaline salts, such as sodium carbonate, are added to yeast fermentations. This causes conversion of intermediate acetaldehyde to acetic acid and ethanol, resulting in formation of increased amounts of glycerol.

According to the fermentation mechanism, one mole of glycerol should be obtained for each mole of acetaldehyde fixed or transformed. Thus, the theoretical yield of glycerol would be 51 g per 100 g of sugar fermented. However, in almost all reports and patents, the maximum yields of glycerol have been of the order of half the theoretical amount. This is accounted for by the fact that even though conditions are quite abnormal for yeast fermentation, the normal alcoholic fermentation continues to an appreciable extent.

The main hindrance to successful exploitation of glycerol fermentations has been difficulty in recovering the glycerol from the fermented beers. In most cases, the concentration of soluble salts exceeds the concentration of glycerol present, even when pure sugar is used as the carbohydrate source. When fermentation of crude substrates, such as molasses, is attempted, the difficulties are enhanced. Recovery of the glycerol from such mixtures is extremely difficult by any of the conventional methods, such as distillation or solvent extraction. Recently, Fulmer, Underkofler, and Hickey[12] have suggested a process involving the use of insoluble sulfites at slightly acid pH which should facilitate recovery.

## SOLUBLE-SULFITE PROCESSES

### The German Process

The process of Connstein and Lüdecke,[5,6] developed to meet emergency requirements of glycerol in Germany during World War I, was utilized by the Protol Gesellschaft in twenty-four factories, producing over 1,000 tons of dynamite-grade glycerol a month.

According to Zerner,[39] the process was also used, to a considerable extent, in Austria. Following the war, Verbeek[36] published accounts of the Protol and Fermentol operations. Foth[11] has also briefly described the process. The German operations were based on the general procedure described in the following.

The mashes consisted of solutions of pure white beet sugar in water. to which nutrient salts and sodium sulfite were added. The proportions were 1 kg sucrose, 50 g ammonium nitrate, 7.5 g dipotassium phosphate, and 400 g sodium sulfite in 10 l water. Magnesium sulfate in small amounts was sometimes also added. To the sugar-salts solution, 100 g of fresh filter-pressed yeast was added and the fermentation allowed to proceed for 48 to 60 hours at 30°C. According to Verbeek, the fermentations were conducted in tanks of 300,000 l (80,000 gal) capacity. According to Henneberg,[16] one plant employed 125,000 gal fermentors and used 60 tons of sugar and 6 tons of yeast daily. In warm weather, it was necessary to cool the fermenting mashes. Much carbon dioxide and hydrogen sulfide were liberated. At the end of the fermentation, the beer was clarified by settling, the clear portion decanted, and the remainder filter pressed. The yeast so filtered off could not be used for seeding fresh mash because of its contamination with so-called wild yeasts. The clear, filtered mash contained 2 to 3% glycerol, 2 to 3% ethanol and about 1% acetaldehyde, in addition to inorganic salts, acetic acid, etc. The filtered beer was distilled to recover alcohol and acetaldehyde, very efficient condensers being required to prevent a great loss of acetaldehyde.

The concentration, purification, and distillation of the glycerol were conducted in central factories. In only one plant was the entire process carried through from fermentation to dynamite glycerol. All the other fermentation plants shipped the glycerol-containing distillation residues to refining centers. From some plants, the thin stillage was shipped. Other fermentation plants concentrated the thin stillage in vacuum evaporators to a density of about 25° Bé and ran it into storage tanks. On cooling, a considerable amount of salt crystallized, mostly sodium sulfite, which was reused in fresh mashes. The crude, concentrated solutions so obtained for the refinery were very impure, brownish-green to yellow in color, saturated with sodium sulfite, and contained about 14 to 18% glycerol.

The analytical yields from normal fermentations, based on the

weight of sucrose fermented, were about 20 to 25% of glycerol, 30% of alcohol, and 5% of acetaldehyde. As far as is known, in the commercial production of glycerol in Germany and Austria, pure sugar was always used as fermentable substrate. There is some indication by Zerner[39] that attempts may have been made to use molasses in Austria, but no record has been found that this was ever employed on a large scale. The recovery of dynamite glycerol from the industrial fermentations rarely exceeded 60% and was usually less than 50% of that present and the recovery problem would have been greatly intensified with the use of molasses, having 30% of nonsugar solids. Tremendous losses occurred in the recovery operations so that usually 10 to 12 kg of refined sugar were required to produce 1 kg of dynamite-grade glycerol in the German practice.

## The Ammonium Sulfite Process

The difficulties in glycerol recovery experienced during operation of the German sodium sulfite process were very great as indicated. If a method could be devised which would materially reduce the content of dissolved solids in the fermented beer, recovery would be facilitated and a fermentation might have more promise. Fulmer, Underkofler, and Hickey[13] attempted to solve this problem by employing ammonium sulfite, which could be removed after fermentation by precipitation and volatilization. In the laboratory work on which the patent of these workers was based, a medium, containing per liter, 150 g sugar (as dextrose), 2.0 to 5.0 g corn steep liquor, 1.5 g ammonium chloride, 0.75 g dipotassium phosphate, 0.75 g monopotassium phosphate, 0.2 g magnesium sulfate hydrate, and 0.1 g calcium chloride, was inoculated with 10% by volume of an active yeast culture. When the fermentation became vigorous, ammonium sulfite was added in 5 g portions, per liter of medium, at about 6-hour intervals until a total equivalent to 60 g of sulfur dioxide had been added. Fermentations under these conditions were extremely erratic. At times, yields up to 20 g of glycerol per 100 g of sugar were obtained, but the usual yields were much lower. It was found that the pH was extremely critical. If the conditions became alkaline, fermentation ceased due to toxicity of free ammonia to the yeast. If the medium became more acid than about pH 6.5, fermentation also ceased because of bisulfite toxicity. By careful control at pH 6.8 throughout, the fermentations were usually

successful, with yields of glycerol in the range of 15 to 20 g per 100 g of sugar, the balance of the sugar being converted to alcohol and carbon dioxide. Later, it was found that fermentations were better if massive inoculations of yeast were employed. Pressed yeast or yeast cultivated by procedures similar to those given in Chapters 9 and 10 may be employed, inoculation being sufficient to give at least 100 million cells per ml of medium. Under these conditions, there is little or no proliferation of the yeast during fermentation and addition of yeast nutrients may be omitted.

A preferred method of operation is as follows: A medium is prepared containing 150 to 200 g of sugar (as dextrose) and 5 g of ammonium sulfite per l. The pH is adjusted to 6.8 by adding ammonia or ammonium hydroxide or sulfur dioxide as required and the medium is then inoculated with a large quantity of yeast sufficient to give a count of more than 100 million yeast cells per ml. The mixture is continually circulated and the temperature maintained at 30 to 32°C. As the fermentation proceeds, acetaldehyde is fixed by the bisulfite present and the medium tends to become more alkaline. The reaction is continuously controlled at pH 6.8 by automatic addition of sulfur dioxide as required by means of a pH recorder-controller. The course of the fermentation is followed by periodic sugar analyses on the medium, and when no further reduction in sugar content occurs, the fermentation is considered finished. The yeast is then centrifuged from the mash and used, together with additional fresh yeast, for inoculation of another batch of fermentation medium.

To the fermented liquor, a slurry of calcium hydroxide in excess of the amount required to make the mixture distinctly alkaline is added. The volatile substances, including ammonia, acetaldehyde, and ethanol, are separated and recovered by distillation. The solids remaining after the distillation, calcium sulfite and excess calcium hydroxide, are removed by filtration or centrifugation. Calcium in solution is precipitated by adding carbon dioxide to the hot solution, and the precipitated calcium carbonate removed by filtration or centrifugation. The remaining liquid contains the glycerol and is relatively free of dissolved solids. From this liquid, the glycerol may be obtained by evaporation and distillation. Or, the liquid may be recycled and used as medium for a new fermentation by adding sugar and ammonium sulfite. In this

way, the glycerol concentration of the beer may be increased, thus facilitating subsequent glycerol recovery.

Although the use of ammonium sulfite as acetaldehyde-fixing agent was successful in the laboratory, very exacting control was necessary to prevent failure and the procedures for eliminating the salts before glycerol recovery are somewhat complicated. No pilot-plant investigation of the process has been attempted and although the process materially reduces the solids content of the fermented beer concentrate, the economics would probably not be very favorable.

## ALKALINE PROCESSES

### The Sodium Carbonate Process

The process developed by Eoff[9] during World War I never attained actual commercial status. However, it was tested, according to the report of Eoff, Linder, and Beyer,[10] in small-plant-scale fermentations at a glycerol refinery with mashes up to 2,000 gal. Eoff made a rather thorough investigation of the fermentation on the laboratory scale before the plant tests. As a result of his experiments he found that addition of various alkaline substances, such as sodium carbonate, potassium carbonate, sodium hydroxide, potassium hydroxide, and borax resulted in increased glycerol yield during the fermentation of sugars by yeast. The carbonates of sodium and potassium gave best results and sodium carbonate was the most satisfactory as to cost and glycerol yield. About 5% of sodium carbonate, based on the weight of the mash, was optimum. Greater amounts stopped fermentation permanently and smaller amounts did not supply sufficient alkali to produce maximum glycerol yields. The sodium carbonate was best added in solid form rather than in solution and in several batches, added as early and as frequently as the yeast would stand it. The most favorable initial sugar concentration was 17.5 to 20 g per 100 ml and the most favorable temperature 30° to 32°C. Under these conditions, on completion, 20 to 25% of the sugar had been converted to glycerol and practically all of the balance of the original sugar to ethanol and carbon dioxide.

Several yeast cultures were tested and one designated *Saccharomyces ellipsoideus* (var. Steinberg) was selected as the best glycerol producer. The yeast was "worked up" by successive trans-

fers into increasing volumes of mash, using 5 to 10% inoculum, and acclimatized to alkali by adding 0.5 to 1% sodium carbonate to the culture mashes.

Details of a typical plant test were as follows. The yeast culture was inoculated into a small volume of sterile grape juice. At the height of vigorous fermentation, 75 ml of the grape juice culture was transferred into 800 ml of sterilized molasses medium of 21.2° Balling. When this was fermenting vigorously, 3 g of soda ash was added and the bottle shaken until solution was complete. When the yeast was most active this was employed as inoculum for a larger volume of molasses mash which was treated at the proper time with soda ash and the development was continued in the same manner until a seed mash of 200 gal was reached. This was used for inoculating the main fermentation mash which contained 4,856 lb of blackstrap molasses and 11 lb ammonium chloride. The final volume was 2,142 gal, and the Balling hydrometer reading was 21.7. The mash contained 16.5 g of sugar per 100 ml by reducing sugar analysis. After inoculation with the seed-yeast culture, the mash temperature was maintained at 32°C, that is, it was reduced by cooling when it rose above this figure. A total of 992 lb of soda ash was added in batches. The percentage of the total soda ash added and the times of the additions were as follows: 12.5% at 2¼ hours, 18.75% at 4½ hours, 25% at 6½ hours, 25% at 10½ hours, 18.75% at 17 hours.

The plant test fermentations required 5 to 7 days for completion. Analysis of a typical beer showed glycerol 3.1%, alcohol 6.75%, sugar 0.86%, and alkali as sodium carbonate 3.6 g per 100 ml. According to Lawrie,[20] Eoff conducted 15,000 gal fermentations at a glycerol refinery in Illinois and obtained glycerol yields of 19.6 to 27.1% from blackstrap molasses, based on fermentable sugar content. Recovery as dynamite-grade glycerol from the fermented beers was only about 50% by direct distillation in a vacuum still.

Although other reports are lacking in the literature, it is known that large pilot plant or semicommercial alkaline fermentations were conducted by different concerns during the 1930's when glycerol was in very scarce supply. Glycerol yields of about 25% were obtainable without difficulty, but, although distinct improvements were made, the problem of recovery was never fully solved.

## The Schade Process

Recently, Schade and Färber[31] and Schade[30] patented fermentation processes in which the volatile constituents, principally ethanol, acetaldehyde, and carbon dioxide are removed during the fermentation by bubbling air, nitrogen, or oxygen through the fermenting medium while the pH is maintained at neutral or slightly alkaline reaction by addition of suitable hydroxides or basic salts. Under these conditions, it is claimed glycerol yields are approximately those obtained by earlier alkaline fermentation processes while the yeast multiplies and is maintained in a healthy state so that it can be reused to ferment fresh amounts of carbohydrates.

In the first process,[31] yeast fermentation in the presence of magnesium carbonate is employed, the reaction being maintained between pH 7 and 8 by addition of 10 to 20% of magnesium carbonate based on fermentable sugar originally present in the medium. In an example, 10 l of hydrolyzed wheat medium, containing 1,700 g of reducing sugars, was added, together with 170 g of compressed yeast of 72% moisture content to a fermentation vat located in a constant-temperature room at 32°C. The vat was equipped with a stirrer, gas disperser, and gas outlets. The medium was aerated at the rate of 1 l per minute. The pH was kept at 7.0 to 7.2 by addition of a total of 170 g of magnesium carbonate. Almost all of the fermentable sugar was consumed in 26 hours and a yield of 310 g of pure glycerol, after refining, and 400 g of yeast containing 72% moisture was obtained.

In the second process,[30] the pH is maintained between 6.9 and 7.3 by the addition of neutralizing agents, such as hydroxides, phosphates, or carbonates of the alkali metals, with aeration to remove volatile products as they are produced. It is stated that 10 to 20% carbohydrate media may be fermented at 34° to 37°C in 12 to 24 hours. In an example, 10 l of hydrolyzed wheat medium containing 1,000 g reducing sugars was mixed with 100 g of compressed yeast of 72% moisture content in a vat and aerated as described before. The pH was maintained at 6.9 to 7.3 by addition of a 5% solution of sodium hydroxide as required. After a period of 12 hours, 795 g of the sugar had been consumed and 150 g of pure glycerol as well as 310 g of yeast containing 72% moisture were obtained. In another example, blackstrap molasses was used as source of carbohydrate and the mash was neutralized during fer-

mentation with a 45% calcium hydroxide slurry. From 494 g of sugar metabolized during the fermentation, 110 g of glycerol were produced.

## INSOLUBLE-SULFITE PROCESSES

About 10 years ago, interest was revived in the production of glycerol from sources other than soap lyes. This resulted in investigation of possible synthesis from petroleum gases which led to a commercial process and to renewed interest in fermentations. In spite of much previous work, fermentation processes had not achieved commercial application, except in Germany during World War I, mainly due to the difficulties in recovering the glycerol from the fermented beers. Since the recovery difficulties were chiefly caused by the high concentration of dissolved solids, if a method could be devised which would materially reduce the content of dissolved solids, recovery would be facilitated and a fermentation process might have promise.

Attention was turned, therefore, at Iowa State College to the possibility of using volatile or insoluble sulfites to furnish bisulfite for fixation of acetaldehyde in yeast fermentations. The extensive work resulted in three doctoral theses and two patents and part of this research has been reported recently.[34,35] The ammonium sulfite process discussed previously was one development from this research.

It was found during this research that such compounds as the removable ammonium sulfite and the insoluble calcium sulfite or magnesium sulfite could be employed, but that the pH must be carefully controlled for success. At too acid reaction, the bisulfite concentration becomes so high that yeast activity is diminished. At low acidity however, the bisulfite concentration is too low to fix acetaldehyde effectively and thus to change the course of the normal yeast fermentation appreciably.

The most satisfactory method for eliminating the necessity of using soluble compounds, which so badly complicate the recovery of the glycerol produced, was found to be by fermentation in the presence of an excess of slightly soluble sulfites. Of those tried, calcium sulfite and magnesium sulfite have given best results, the second being preferred.

The fixation of acetaldehyde, with resultant increase in glycerol

production by yeast, depends on the presence of bisulfite ions in the medium. However, excess of bisulfite is toxic to yeast and is, therefore, detrimental to yeast fermentation. With slightly soluble sulfites, the bisulfite-ion concentration is dependent on the solubility of the sulfite and the pH of the solution due to the equilibria:

$$MgSO_3 \text{ (solid)} \rightleftharpoons MgSO_3 \text{ (dissolved)} \rightleftharpoons Mg^{++} + SO_3^{=}$$
$$SO_3^{=} + H^{+} \rightleftharpoons HSO_3^{-}$$

Increase in the acidity, that is, lowering the pH, not only increases the bisulfite concentration but also, by mass action, increases the total solubility of the slightly soluble sulfite employed.

Early laboratory work with fermentations, employing slightly soluble sulfites, was conducted with a semisynthetic medium containing sugar and yeast nutrients. A typical medium had the composition per liter of 150 g dextrose, 3.5 g Difco yeast extract, 1.5 g ammonium chloride, 0.75 g dipotassium phosphate, 0.75 g monopotassium phosphate, 0.4 g magnesium sulfate heptahydrate, and 0.1 g calcium chloride. Excess solid calcium sulfite or magnesium sulfite was added, the pH adjusted to various values between 4.0 and 7.5 by addition of the required amounts of hydrochloric acid or potassium hydroxide, and inoculated with an active culture of distillery yeast. Fermentations occurred in the pH range of 4.5 to 7.5 with calcium sulfite and of 5.0 to 7.5 with magnesium sulfite. Glycerol yields were highest with the lowest pH values that were tolerated by the yeast and were considerably better with magnesium sulfite than with calcium sulfite. This is undoubtedly due to the somewhat greater solubility of magnesium sulfite than of calcium sulfite, furnishing a higher concentration of bisulfite for acetaldehyde fixation. Subsequent work showed that if the reaction was carefully controlled at the optimum pH level throughout the fermentation, rather than merely adjusted at the beginning of fermentation, more satisfactory glycerol yields were obtainable with either calcium sulfite or magnesium sulfite, although the second gave slightly better, more uniform and consistent results.

Examples of the laboratory work on which the patent was based are as follows. To 1,500 ml of medium of the given composition was added 420 g of magnesium sulfite hexahydrate and a yeast culture which had been grown in the presence of magnesium sulfite. The mixture was incubated at 30°C while the reaction was controlled at pH 6.2 by automatic addition of 50% acetic acid as

required. The fermentation was complete in less than 72 hours and analysis showed a yield of 23.15% glycerol on the weight of the dextrose originally present.

In another example, 1,550 ml of medium of the composition described was inoculated with an acclimatized inoculum consisting of calcium sulfite and yeast. This was incubated at 30°C and the pH regulated at 5.0 by automatic addition of sulfurous acid as required. After fermentation for 72 hours, the yield of glycerol was 21.5%.

Obviously, the control of the pH by adding conventional acids, such as mineral acids or acetic acid, results in formation of soluble salts of magnesium or calcium in the fermented beers, which defeats the purpose of decreasing the soluble solids content. Likewise, yeast proliferation is slow in the medium containing the sulfites. Modifications were, therefore, made, employing massive yeast inoculation and continuous control of pH by addition of sulfur dioxide as required. In a medium containing 15 g dextrose and 60 g magnesium sulfite tetrahydrate per 100 ml, with pH adjusted to 6.5 and inoculated with pressed yeast to a count of 150 million cells per ml, fermentation was vigorous and after 72 hours, the glycerol yield was 22% of the original weight of dextrose.

Based on the laboratory work, a preferred method of operation is as follows. A medium is prepared, containing 150 to 200 g sugar (as dextrose) and an excess of magnesium sulfite (e.g., 200 g of the hydrate) per liter. The pH is adjusted to 6.5 by addition of sulfur dioxide and the medium is inoculated with a large quantity of yeast sufficient to give a count of more than 100 million yeast cells per ml. The mixture is continuously agitated and the temperature maintained at 30° to 32°C. As the fermentation proceeds, acetaldehyde is fixed by the bisulfite present and the medium tends to become more alkaline. The reaction is continuously controlled at pH 6.5 by automatic addition of sulfur dioxide as required. At the end of the fermentation period, the mash is filtered or centrifuged to remove the excess of magnesium sulfite and the yeast. These may be reused in a new fermentation. To the filtrate a magnesium hydroxide slurry is added in excess of the amount required to make the mixture distinctly alkaline. The volatile substances, acetaldehyde and ethanol, are separated and recovered from the mixture by distillation. During this process, the acetaldehyde-bisulfite complex is decomposed and magnesium sulfite precipitated. The sulfite is

filtered from the hot solution and there remains an aqueous solution containing the glycerol and relatively small amounts of soluble salts. The solution may be treated to recover the glycerol by distillation or solvent extraction, or may be refermented to increase the glycerol content by adding more sugar, sulfite and yeast before recovering the glycerol. Since most of the magnesium sulfite and yeast are recovered for reuse, the process should have industrial value.

Application for patent was made and a patent was granted to Fulmer, Underkofler, and Hickey.[12] A license was issued to a commercial fermentation concern with the plan of conducting pilot-plant investigations to evaluate the practical and economic possibilities of the process for industrial exploitation. It was planned to use high-test molasses as a fermentation substrate, but about that time, importation of molasses was stopped due to the shipping shortages of World War II. This commercial concern then investigated the possibility of adapting the process to malt-saccharified grain mashes. The results were very disappointing. The fermentations of filtrates from grain mashes saccharified with malt were extremely sluggish and glycerol yields very poor. As a result, the license agreement was cancelled.

Subsequent extensive investigations at the Iowa State College[35] disclosed that maltose is fermented very slowly by yeast in the presence of sulfites or alkalis. The alkaline fermentation medium employing sodium carbonate, media containing insoluble magnesium sulfite or calcium sulfite, and media containing soluble sodium sulfite or ammonium sulfite all gave prolonged, sluggish fermentations when maltose was the carbohydrate source. On the contrary, glucose, fructose, and sucrose all were fermented rapidly in similar media, with good yields of glycerol. No satisfactory explanation of the abnormal behavior of maltose has been found, but it may be related to possible difference in the mechanism of fermentation of this sugar. Stark and Somogyi[32] have reported that maltose is not fermented satisfactorily under alkaline conditions. From the extensive experimental work, the conclusion was reached that the insoluble-sulfite process is applicable only to fermentations of solutions containing glucose, fructose, or sucrose. The most favorable raw material other than pure sugars would undoubtedly be high-test molasses which has a high sugar content and relatively low amounts of nonfermentable solids.

However, it has been found that acid-hydrolyzed starch mashes might also be a satisfactory substrate. An investigation has been made at the Iowa State College on the glycerol fermentation of mashes prepared by acid hydrolysis of corn starch and of the starch slurry resulting from the separation of gluten from wheat flour for the manufacture of monosodium glutamate. The starch slurry, containing approximately 30 g of starch, was mixed with an equal volume of dilute sulfuric acid, and cooked under steam pressure, with conditions optimum to secure maximum production of fermentable sugars. The acid concentration, temperature and time are interrelated variables. With the laboratory equipment available, the optimum conditions chosen for hydrolyzing 15% starch slurries were heating at 15 psi steam pressure for 4 hours with 0.15 *N* sulfuric acid. In commercial practice, of course, higher steam pressures for shorter periods would be preferable. For example, Ruf, Stark, Smith, and Allen[29] have described a procedure for the large-scale preparation of fermentation mashes by acid hydrolysis of grains. The hydrolyzed starch mashes were fermented in the laboratory, after addition of 20 g per 100 ml of magnesium sulfite tetrahydrate and massive inoculation with yeast to give at least 150 million yeast cells per ml. The fermentations were incubated at 30°C and pH was controlled at the optimum value of 6.5. Glycerol yields were 29%, based on the weight of the original starch present.

A process somewhat similar to that of Fulmer, Underkofler, and Hickey has apparently been operated successfully on molasses in South Africa. According to Duchenne,[8] a 21° Brix sirup was fermented for 5 days at 35°C with *S. ellipsoideus* in the presence of an acid slurry of calcium sulfite and bisulfite which was added in increasing amounts and with continuous slow stirring during the fermentation. The calcium sulfite was removed by settling, the liquor neutralized with calcium hydroxide slurry, filtered, concentrated to a thick sirup, and distilled with steam. Yields based on the weight of sugar were reported as 9.8 to 13.4% alcohol and 10.1 to 15.3% refined glycerol, depending on the amount of sulfite used. The necessary sulfite could be obtained as a by-product from the juice-purification process in sulfitation sugar factories. It was stated that a sugar factory producing 8,000 tons of molasses a year can manufacture 6 tons of glycerol and 1,000 gal of alcohol a day by the process.

## BACTERIAL PRODUCTION OF GLYCEROL

Recently, it has been discovered that certain strains of *Bacillus subtilis* produce appreciable amounts of glycerol. Neish, Blackwood, and Ledingham[24,25] first noted the production of glycerol by Ford's strain of *Bacillus subtilis.* This organism produced various products, depending on environmental conditions, including besides other minor substances, 2,3-butanediol and acetoin, glycerol, ethanol, lactic acid, and carbon dioxide. Under anaerobic conditions, 2,3-butanediol and glycerol were the main products in the fermentation of mashes containing 3% dextrose, 1% Difco yeast extract and 1% calcium carbonate at 30°C and pH 6.2 to 6.8. Under these conditions, the yields of the two major products, based on the weight of glucose, were 28.2% of 2,3-butanediol and 20.4% of glycerol. The authors state: "It is quite probable that this glycerol fermentation may be of industrial importance if active organisms giving high yields are obtained. Although the fermentation will require close control, it should be more satisfactory than glycerol production from yeast using the sulfite or alkaline process."

Later, Blackwood, Neish, Brown and Ledingham[2] investigated a considerable number of strains of *Bacillus subtilis*, especially studying the fermentation characteristics of six named strains and twenty-seven isolates. The medium employed contained, per liter, 50 g glucose, 5 g Difco yeast extract, 0.5 g monopotassium phosphate, 0.5 g dipotassium phosphate, 0.2 g magnesium sulfate hydrate, and 10 g calcium carbonate. The medium was inoculated with 7.5% of a 24-hour inoculum grown at 37°C. Extreme variations in the yield of the various products were obtained with the different strains. With some strains, up to 86% of the glucose was fermented in accordance with the equation:

$$3\ C_6H_{12}O_6 \longrightarrow 2\ CH_3{\cdot}CHOH{\cdot}CHOH{\cdot}CH_3 +$$
$$2\ CH_2OH{\cdot}CHOH{\cdot}CH_2OH + 4\ CO_2$$

The highest-yielding strain gave yields, based on sugar weight, of 27.4% 2,3-butanediol, 29.4% glycerol, 2.0% ethanol, 11.5% lactic acid, and 28.1% carbon dioxide. The fermentations were run anaerobically, with nitrogen gas bubbled through the medium. The fermentation of the glucose in 5% concentration was 99.5% complete in 8 days at 34°C.

Neish, Ledingham, and Blackwood[26] obtained a patent claiming

the process for obtaining glycerol by fermentation, involving inoculating a sterile 5% solution of sugar, together with essential nutrients, with a special strain of *B. subtilis* and fermenting at 37°C in the presence of 1% calcium carbonate which is kept in suspension until all the sugar is utilized. Fermentation products as lb per 100 lb of sugar are: glycerol, 29.4, 2,3-butanediol 28.1, lactic acid 11.6, ethanol 2.2, formic acid 0.3, and carbon dioxide 36.4.

By comparison with the sulfite yeast processes, it would seem that the glycerol yields produced by the bacteria are quite similar. However, the principal by-products are 2,3-butanediol, lactic acid, and ethanol from the bacterial fermentation, rather than acetaldehyde and ethanol from the yeast process. Before the process could be considered for industrial use, considerable research would be necessary to develop procedures for satisfactorily fermenting higher substrate concentrations in reasonable time. Much research might be required on methods for recovering and separating the relatively non-volatile compounds produced in large amounts, the 2,3-butanediol and lactic acid, from the glycerol. Markets for the 2,3-butanediol would also be necessary, since this is produced in an amount almost equal to that of the glycerol.

## COMPETITIVE PROCESSES

As has been mentioned previously, the principal commercial source of glycerol has always been soap making which yields glycerol as a by-product. The synthesis of glycerol from the petroleum gas, propylene, was developed on the pilot-plant scale by Williams[38] and an industrial plant employing the process was constructed in 1948.[1] Announcement was made in December 1950 that it was expected to expand the synthetic process by about 50% during 1951, increasing the production of synthetic glycerol from the then estimated 35 million lb a year to 52 or 53 million lb. Total annual production of glycerol in 1949 in the United States was about 194 million lb, crude basis, exclusive of imports. Glycerol supplies continue to be subnormal despite increased by-product recovery in saponification operations, increased synthesis, and greater imports. Conditions remain favorable for introduction of a glycerol-fermentation process, but at the present time, there is apparently little or no activity in this field by industrial-fermentation concerns.

## LITERATURE CITED

1. Anon., *Chem. Eng.*, **55**, No. 10, 100, 134 (1948).
2. Blackwood, A. C., A. C. Neish, W. E. Brown, and G. A. Ledingham, *Can. J. Research,* **25B**, 56 (1947).
3. Cocking, A. T., and C. H. Lilly, U. S. Patent 1,424,838 (1922).
4. Connstein, W., and K. Lüdecke, *Ber.*, **52**, 1385 (1919).
5. Connstein, W., and K. Lüdecke, U. S. Patent 1,368,023 (1921).
6. Connstein, W., and K. Lüdecke, U. S. Patent 1,511,754 (1924).
7. Cornée, C., French Patent 865,691 (1941).
8. Duchenne, J. O., *Proc. 16th Ann. Congr. S. African Sugar Tech. Assoc.*, **1942**, 45.
9. Eoff, J. R., U. S. Patent 1,288,398 (1918).
10. Eoff, J. R., W. V. Linder, and G. F. Beyer, *Ind. Eng. Chem.* **11**, 842 (1919).
11. Foth, G., *Handbuch der Spiritusfabrikation,* Berlin, Paul Parey, 1929.
12. Fulmer, E. I., L. A. Underkofler, and R. J. Hickey, U. S. Patent 2,388,840 (1945).
13. Fulmer, E. I., L. A. Underkofler, and R. J. Hickey, U. S. Patent 2,416,745 (1947).
14. Grover, C. E., U. S. Patent 2,430,170 (1947).
15. Haehn, H., U. S. Patent 2,189,793 (1940).
16. Henneberg, W., *Handbuch der Gärungsbacteriologie,* Vol. II, p. 385, Berlin, Paul Parey, 1926.
17. Hesse, F. M., *Rev. Cubana Azucar y Alcohol,* **1**, 282 (1935).
18. Hodge, H. M., U. S. Patent 2,381,052 (1945).
19. Krug, W. F., and F. A. McDermott, U. S. Patent 1,990,908 (1935).
20. Lawrie, J. W., *Glycerol and the Glycols,* New York, Reinhold, 1928.
21. Leffingwell, G., and M. Lesser, *Glycerin, Its Industrial and Commercial Applications,* New York, Chemical Publishing, 1945.
22. May, O. E., and H. T. Herrick, *Ind. Eng. Chem.*, **22**, 1172 (1930).
23. McDermott, F. A., U. S. Patent 1,725,363 (1929).
24. Neish, A. C., A. C. Blackwood, and G. A. Ledingham, *Science,* **101**, 245 (1945).
25. Neish, A. C., A. C. Blackwood, and G. A. Ledingham, *Can. J., Research,* **23B**, 290 (1945).
26. Neish, A. C., G. A. Ledingham, and A. C. Blackwood, U. S. Patent 2,432,032 (1947).

27. Neuberg, C., J. Hirsch, and E. Reinfurth, *Biochem. Z.*, **105**, 307 (1920).
28. Pasteur, L., *Compt. rend.*, **46**, 857 (1858).
29. Ruf, E. W., W. H. Stark, L. A. Smith and E. E. Allen, *Ind. Eng. Chem.*, **40**, 1154 (1948).
30. Schade, A. L., U. S. Patent 2,428,766 (1947).
31. Schade, A. L., and E. Färber, U. S. Patent 2,414,838 (1947).
32. Stark, I. E., and M. Somogyi, *J. Biol. Chem.*, **142**, 579 (1942).
33. Tomoda, Y., *J. Soc. Chem. Ind. (Japan), Suppl. binding*, **31**, 151B (1928).
34. Underkofler, L. A., E. I. Fulmer, R. J. Hickey, and T. M. Lees, *Iowa State Coll. J. Sci.*, **26**, 111 (1951).
35. Underkofler, L. A., E. I. Fulmer, T. M. Lees, and P. H. Figard, *Iowa State Coll. J. Sci.*, **26**, 135 (1951).
36. Verbeek, P., *Zeifensieder-Ztg.*, **48**, 591, 633 (1921).
37. Whalley, M. W., *Abstracts of Articles and Patents on the Production of Glycerol by Fermentation*, Ottawa, Canada, National Research Council of Canada, N. R. C. No. 1040 (1942).
38. Williams, E. C., *Chem. & Met. Eng.*, **48**, 87 (1941).
39. Zerner, E., *Ber.*, **53B**, 325 (1920).

# Part II. THE PRODUCTION OF YEAST

CHAPTER 9

# COMMERCIAL YEAST MANUFACTURE

*Roy Irvin*

The art of preparing yeast-leavened bread dough is very ancient. Its origin might be in the fact that a mixture of grain meal and water is likely to start fermenting if let stand long enough to be inoculated with wild yeast. The fermentation once started, propagation and preservation of favorable cultures could be continued by reserving a small portion of dough to initiate fermentation in the next batch. A culture maintenance could be established also by transfers from fermenting grain mashes or grain infusions. The term "starter," referring to pieces of old dough, fermenting potato water, etc., was known to our grandmothers. Later, yeast in a more concentrated form was derived as by-product from the distilleries. The excess water was pressed from the yeast and the product was sold to nearby bakeries. Many attempts were also made to utilize waste yeast from breweries, but due to its inferior action in bread dough and its poor keeping quality, brewers' yeast did not prove very satisfactory in the bake shop. Considerable investigational work was also done on the conditioning or treatment of waste brewers' yeast to make it suitable for baking purposes, but the results were not generally acceptable.

Bakers' compressed yeast at first was mostly a by-product of

the distilleries where the main interest lay in alcohol or vinegar production. There were, however, some early efforts in this country to improve yeast-making methods. Terry[108] in a patent issued in 1869 claimed the use of a grain mash soured by lactic acid fermentation as a yeast propagation medium. A patent to Fleischmann[33] in 1870 described a grain mash and gave details for carrying out the "skimming" or Vienna process of yeast making. Fleischmann inoculated his wort with brewers' yeast to start fermentation. Levy[67] modified the Vienna process by adding fresh mash as the fermentation weakened, an improvement later developed into the "Zulauf" procedure. Rainer[84] in 1879 patented the separate feeding of sugar and nitrogenous materials to the fermentor, the use of dilute solutions, and a continuous method of operation for the production of yeast withut appreciable alcohol formation.

A typical procedure[114] for making baker's yeast according to the Vienna or skimming method was as follows: A mash of 50% corn, 30% rye, and 20% malt (for saccharifying) was prepared and from it a wort of about 10 to 11° on the Balling saccharometer scale was obtained. This was fermented for about 48 hours without cooling or aeration, at which time, the saccharometer showed about 1°. At 20 hours, however, the yeast had risen to the top of the fermentor as a foamy mass and was considered "ripe" and ready for skimming. It was passed through silk screens to remove grain solids, diluted with water, and allowed to settle. After three or four washings made in a similar manner and a total washing period of 20 to 24 hours, the settled yeast was collected and pressed. The yield was 11.5 to 12.5%, based on the quantity of dry grain taken for the mash. Alcohol recovered after 48 hours of total fermentation time amounted to approximately 15 qt, 100° proof per bushel of grain.

Muspratt's handbook,[76] 1915, is the authority for the following observations on the transition from the older to the more modern yeast-manufacturing methods. Aeration of the nutrient medium for growing yeast was established at Rainer's time, 1879, and was covered by a patent to Marquardt in that year. It made little advancement in Germany until 1889, but was employed earlier in England, Sweden, and Denmark. Its most successful application, however, was not attained until the souring of mashes with a lactic acid ferment was developed. The yield of pressed yeast was increased from the 12 to 13% of the old Vienna process to 18 to 22% by the new process of aeration. Braasch in 1908 improved

production methods by using low seeding, low temperature, longer times, and limiting alcohol formation during fermentation. He attained yields of 30 to 35%. While Braasch's yields were considered high, his low dilutions during fermentation, 2 to 3° Balling at the start, called for the use of more water and air. These facts, together with a loss in alcohol yield, must have been considered as unfavorable. In 1915, a yield of 40% pressed yeast was considered quite satisfactory in Germany. Higher yields were not wanted, because a favorable price for alcohol prevailed and made the dual-purpose fermentation quite economical.

Muspratt's handbook states further that distillers' slop was used, to some extent, in yeast growing. The possible need for mineral salts when yeast yields were increased was observed, and molasses was used as a nutrient medium, especially in Austria and Hungary. Mixed mashes of molasses and malt sprouts were subjected to a lactic acid fermentation in the preparation of worts for yeast growth. It was recognized that yields and quality of yeast were dependent on strain characteristics to a considerable extent. Numerous other factors, including growth conditions and the influence of natural food materials and pure chemical compounds on yeast propagation, also were investigated.

In the United States, the manufacture of yeast for baking is limited to a rather small number of companies with yeast usually as the sole or principal product. The simultaneous production of alcohol and yeast is not profitable under present economic conditions, since in this country, competition is quite keen in both the yeast and distilling industries and the types of raw materials, plant locations, and other economic considerations usually favor the separation of the two industries. It is true that some American yeast companies developed from breweries or distilleries, but all now operate on the same basis in regard to raw materials, general plant methods, and types of yeast produced. The yeast business is small in comparison to many other industries, but is highly essential as can be readily understood.

In 1944, according to the Federal Trade Commission, over 204,000,000 lb of yeast were sold to bakers in the United States and 12,000,000 lb were purchased by the government, presumably for making bread for the armed forces. A smaller amount was also sold in the retail market for home baking. The U. S. Department of Commerce[111a] reported the following comparative figures which

indicate the growth of the yeast industry during the period 1939-47. In 1939, 219,364,000 lb of yeast were manufactured with a value of \$16,418,000 and in 1947, the estimates were 270,104,000 lb valued at \$43,884,000. In the 1947 production figure, however, was included active dry yeast calculated on the basis of 1 lb of dry yeast being equivalent to 2 lb of compressed yeast. Since considerable active dry yeast was made in 1947, it is evident that the figure of 270,104,000 lb does not represent total equivalent of pressed yeast production.

## RAW MATERIALS AND THEIR PREPARATION

In the United States, yeast-making methods have tended to become standardized within certain limits. The use of cheap raw materials, like molasses and inorganic yeast nutrients, the employment of aeration, the feed-in or "Zulauf" system, suitable yeast strains, and pure-culture methods have been adopted by all successful manufacturers for economic reasons. About the beginning of this century, however, when improvements over the old Vienna method were made, various raw materials for yeast growing were investigated. New sources of carbon and nitrogen for yeast-cell growth were sought and there was considerable controversy as to the availability to yeast of the carbon in nonsugar organic compounds, like organic acids and alcohol, and the relative merits of organic and inorganic nitrogen sources. In this earlier work, information on the essential growth factors for yeast was lacking and, as a consequence, erroneous and conflicting conclusions often were formed on the value of new and cheaper raw materials.

World War I brought high grain prices and forced yeast makers to use more molasses as a source of sugar. In Germany, Claassen was much concerned with what he considered the inadequacy of an all molasses-salts mash and patented[18] a process for making yeast from molasses that called for varying amounts of organic nitrogen, derived from malt and malt sprouts and inorganic nitrogen, each added in successive stages of yeast propagation. A patent in 1930 to Bratton,[13] in which the use of a setting wort, high in assimilable proteins, followed by a feed wort, high in sugar, was claimed, was based on a similar conception of the need for more organic nitrogen than molasses contains. Though Hayduck claimed, in his various patents, successful results with the mineral salts-molasses process, the industry was slow to eliminate supplementary sources of organic nitrogen. With newer knowledge and prevailing high prices for

grains, yeast makers, however, have now learned how to produce good yeast from relatively inexpensive raw materials, such as beet and cane molasses and inorganic compounds.

Beet and cane molasses contain about 50% sugar with a variation of a few percent above or below this level. Raffinose, in varying amounts, occurs in beet molasses and is not readily utilized by yeast. A distinction is made between "total" and "fermentable" sugar and the difference may amount to several percent, depending on how each type is determined. The yield of yeast, moreover, is not always closely related to the sugar content of the molasses, indicating a variation in growth factors or trace elements which influence yeast growth independently of the sugar.

Both cane and beet molasses supply not only sugar, but also other yeast nutrients. Beet molasses is higher in nitrogenous compounds than cane molasses and also contains an adequate supply of potassium. Both vary in composition according to their source; beet molasses from the central states is higher in nitrogen than that from the irrigated lands in the western states, and cane molasses also exhibits regional influences. Refiners' blackstrap, a by-product of sugar refineries, is especially desirable because it contains less gummy material than sugar-house blackstrap and is consequently easier to process and filter. Beet molasses gives very little filtration difficulties. Yeast makers prefer beet to cane molasses, or a mixture of the two, to cane alone. Since molasses is the principal raw material in bakers' yeast manufacture, price considerations determine the type of molasses employed to a large degree.

Although beet molasses is higher in nitrogenous substances than cane, the second is richer in certain growth factors which are important in producing good yields of yeast. The following data, reported by Rogers and Mickelson,[88] show the relative quantities of a number of vitamins in the two kinds of molasses.

*Micrograms per Gram Molasses*

| | Thiamin | Riboflavin | Nicotinic acid | Pantothenic acid (Ca Salt) | Folic acid | Pyrodoxine (as HCl) | Biotin |
|---|---|---|---|---|---|---|---|
| Beet Molasses | 1.3 | 0.41 | 51.0 | 1.3 | 2.1 | 5.4 | 0.053 |
| Cane Blackstrap | 8.3 | 2.5 | 21.0 | 21.4 | 0.038 | 6.5 | 1.2 |

Except for nicotinic and folic acids, the cane product is better supplied with growth stimulants than beet molasses. However, the

so-called "high-test" cane molasses, though relatively higher in sugar than blackstrap, is lower in ash and growth-promoting substances.

The total nitrogen of beet molasses amounts to about 2% or less. Claassen believed that 50% of the nitrogen was assimilable by yeast, while Wendel held that 28% and Kellner suggested that 66% was available.[17] Betaine, which occurs in considerable amount in beet molasses, was considered by Claassen to be only slightly assimilated by yeast. In practice, the yeast maker can assume that around 0.3 to 0.6% of raw beet molasses is available nitrogen and can make his calculation accordingly. The exact figure will be determined by experience.

Most fermentable carbohydrates could conceivably be employed for yeast propagation and many diverse sugar-containing products have been used for the purpose, or such application has been suggested in the literature. Not many of them, however, are of industrial importance. Sugar from grains, potatoes, etc., can no longer compete with cheaper molasses. One possible exception is corn molasses, or hydrol, a by-product of corn processing plants. It has the disadvantage of a high content of sodium chloride (around 5 to 6%) and is comparatively poor in yeast-growth factors.

In modern practice, the principal sources of nitrogen for yeast growth are the assimilable nitrogenous compounds in molasses and inorganic ammonium compounds. Corn steep-water concentrate is also frequently employed as a supplier of nitrogen. In addition to available nitrogen, steep water furnishes valuable salts and growth stimulants. Many other substances have been mentioned in the literature, or claimed in patents, as yielding yeast-assimilable nitrogen. Among the products covered by patents are hydrolyzed packing-house by-products, such as keratin, elastin, and collagen;[104] urea as a substitute for ammonium compounds;[29] vegetable proteins peptonized with the proteases of bacteria;[27] carbamide;[4] Steffens waste water;[23] distillery slops;[57] hydrolysates prepared by the acid digestion of such substances as leguminous flours, oil cakes, seeds, bran, dried yeast, casein, meat meal, and fibroin;[82] urea plus a source of urease, such as soybean meal;[35] and the ureides of glucose, maltose, etc.[12] This list does not at all cover the field of suggested nitrogen sources, but it does indicate the wide variety of products employed. Before molasses came into general use, grain mashes, supplemented with malt and malt sprouts, supplied the necessary nitrogen for yeast. Now ammonium compounds, such as the sulfate

or phosphate, and aqua ammonia are the preferred sources, the quantity taken for mashing and fermenting being calculated to supply the deficiency of assimilable nitrogen in the other mash materials. Control of pH during fermentation can be effected also by the proper ratio of ammonium sulfate or phosphate to ammonia.

Claassen[17] reported that the assimilation of ammonia nitrogen by yeast is dependent on the reaction of the nutrient medium, stating that at low acidity, 90.5% of the ammonium radical is utilized, while only 76.6% at high acidity. Wohl[117] quoted Wendel to the effect that a low level of nitrogen in the medium limits both the yield of yeast and the quantity of nitrogen in the yeast itself, but that above a certain level, the yield remains constant while the percentage of nitrogen in the yeast cells increases. Fulmer,[37] working with synthetic media, concluded that the pH of the solutions was not a factor in the effectiveness of the ammonium ion on yeast growth, but that temperature was of significance. He offered the following equation as showing the influence of temperature on the quantity of ammonium chloride required for maximum yeast growth:

$$\text{Normality of } NH_4Cl \text{ for maximum growth} = 0.00057t + 0.0179$$

where $t$ = temperature. Fulmer also stated that the anion of the ammonium compounds was not important. In commercial practice, however, it is customary to supply only sufficient ammonia in one form or the other to produce the nitrogen level desired in the finished yeast.

Phosphorus is as essential for normal yeast activity and growth as nitrogen, and is partly supplied as phosphoric acid or its salts, the quantity depending on the $P_2O_5$ level desired in the finished yeast and the amount available in the other raw materials. A supplement of the alkaline earths, calcium and magnesium, may be advisable where the water and raw materials are abnormally low in these elements. Potassium is usually present in sufficient quantity in most molasses mashes. Williams[115] employed in a synthetic medium for yeast, in addition to the ordinary elements: nitrogen, oxygen, phosphorus, carbon, potassium, magnesium, calcium, sulfur and chlorine, the following trace elements: thallium, manganese, boron, iron, copper and iodine.

When only grain materials were used for making bakers' yeast, a wort was prepared by saccharifying the cooked grains with either

dry or green (not dried) malt. It was common practice to add malt sprouts to the mash bill, the sprouts supplying valuable assimilable nitrogen and growth stimulants as well as a good filtering bed in the lauter tub. After the heavy wort drained off, sparging with hot water was started and continued until the effluent was practically free from dissolved solids. The spargings were frequently used to set the fermentor and the heavy wort added later, if the feeding-in process was followed. By the time high dilutions and aeration were adopted, souring of the mash before filtration with a lactic organism, such as *L. delbruckii* at a temperature of around 50°C, was the usual practice. At a later date, molasses was added to the mash before souring and the excess acid was neutralized with ammonia, permitting the lactic acid organism to continue to function and not be killed by its own by-product.

In modern practice, wort is usually made by dilution of molasses with water, acidifying, heating, and filtering. Sulfuric acid, phosphoric acid, superphosphate, and ammonium salts are some of the common chemicals employed in the mash preparation. The choice of treating chemicals, dilution, pH, time and degree of heating, depend on the kind of molasses used and on working conditions in the plant. If for example, the treated mash is clarified by settling and the finished wort decanted, superphosphate can be employed to advantage. Beet-molasses mashes are clarified fairly well by centrifuging, which is less satisfactory for cane mashes. Filters of the plate and frame variety or of the Sweetland type, are particularly suited to molasses clarification when used with filtration aids like filter cell. The clarified wort is usually stored hot to avoid infection. Wooden tubs are well adapted to mashing and wort storage, though subject to higher maintenance cost than metal tanks. Steel tanks are unsuitable because of the corrosive action on iron of the hot and slightly acid wort.

Beet molasses may frequently be high in sulfite which can be reduced in quantity by aeration of the diluted and acidified molasses wort at elevated temperatures. Though growing yeast can tolerate a certain amount of sulfite compounds, excess quantities are harmful. Claassen believed that 0.35% sulfur dioxide in the raw molasses was not harmful to either yeast yield or quality.

Improvement in molasses defecation has been given considerable attention by yeast technologists. Bennet and Peake[7] made a molasses mash containing 15% ground nut cake and, after filtration, added

tannin and refiltered. Wohl[116] patented a process for clarifying and sterilizing molasses in the cold by the use of mineral acids. Corby, Scales, and Buhrig[22] first made alkaline a diluted cane-molasses solution, then nearly neutralized with phosphoric acid, and finally heated and filtered the treated mash. Another modification[39] called for dilution of beet molasses and treatment with a soluble alkali silicate followed by addition of a base from the alkaline-earth group to form a coarse precipitate. Hoffman, Frey, and Hildebrandt[50] also employed silicates to clarify cane molasses on the alkaline side and in one variation of this process, first treated the wort with ammonia and gelatin and then with silicates. Roth[91] claimed improvement over the customary hot-acid treatment of diluted molasses by following the first treatment and filtration by further dilution, acid additions, and agitation to coagulate colloidal substances not removed by the first operation. The same theory as to the value of a double dilution was the basis of a patent to Steinacker[106] who worked with the centrifuge. He clarified molasses first at 40° to 50° Balling and then at 15° to 20° and claimed that some substances soluble at the higher wort concentrations were made insoluble by the second dilution. Clarifications by precipitating calcium sulfate in the molasses wort was the subject of a German patent.[78] Buhrig and Harff,[15] working with cane-molasses worts, preferred acid treatment (pH 4 to 6) to the alkaline reaction and after diluting to 30° to 50° Balling, heated their wort under 10 lb pressure for 30 minutes. A clear solution was said to be obtained by settling and decanting after 10 to 12 hours. Mead[72] clarified cane molasses with pyrophosphate. Bleaching of a diluted and acidulated molasses by direct current at a density of one ampere per square foot was patented by Thompson and Hinchley.[109]

## PURE-CULTURE MAINTENANCE

Because of the high quality and uniformity now demanded in bakers' yeast, it is imperative to select suitable cultures of *S. cerevisiae* and maintain them in a pure condition for making seed yeast. Appropriate laboratory equipment and trained personnel must be available to assure that the starting inoculum for the plant pure-culture department is on hand when needed. It is customary for the laboratory to carry the selected culture on solid or liquid media and to make transfers to suitable flasks which can, in turn, be used for aseptic inoculation of the pure-culture apparatus.

For the isolation of single yeast cells to start new pure cultures, Prescott and Dunn[83] mention the following methods: According to the Hansen procedure, a dilute suspension of yeast in gelatin is spread on a ruled cover slide which is inverted over a moist chamber. Single cells are located with the microscope and colonies developed from them are later picked to start growth in culture tubes. Lindner's hanging-drop method is similar except that individual droplets of a dilute yeast suspension in liquid medium are placed on a cover glass before it is inverted over the moist chamber. Picking colonies from agar plates is a third working method, but it is less reliable because any given colony may have started from more than one yeast cell. A fourth and quite satisfactory technique is the micromanipulator procedure by which any cell seen under the microscope can be isolated and at once placed in nutrient medium for development. For the identification and characterization of strains of *S. cerevisiae,* useful criteria are cell size and shape, appearance of giant colonies, budding and spore-forming characteristics, and the ability to ferment various sugars. Yeast strains suitable for bread making, however, cannot be properly evaluated without actual baking tests.

Nutrient media and conditions for carrying pure cultures in the laboratory probably vary from plant to plant. According to one patented culturing method,[61] improvement is claimed by not allowing the yeast to exhaust the sugar of the medium and be subjected to a high alcohol concentration. Frequent transfers from only partially fermented culture tubes are said to produce superior seed yeast. Fabian and Wickerham[31] found that certain characteristics of some yeasts are less stable when they are cultured in cider and malt extract than when they are grown in synthetic media. This observation might be of interest to yeast manufacturers, since malt sirup is frequently employed as a yeast-culturing medium.

Final propagation by the laboratory of the chosen yeast strain is made in a Pasteur flask, or some modification of it, the selected flask usually having a delivery tube that can be aseptically attached to the first unit of the plant pure-culture station. Treatment of the connecting surfaces of the equipment at the time of inoculation with alcohol or by flaming is good practice.

## CONTAMINATION PROBLEMS

The cultivation of microorganisms on a large scale is beset with many contamination problems and yeast manufacture is no exception. Fortunately, however, yeast is a hardy and fast-growing organism and will tolerate measures that cannot be applied to the propagation of certain other fungi or bacteria. Yeasts are not injured seriously by amounts of chlorine that would kill bacteria [59,65] and will withstand relatively high acidities. Yeast cream from the separator can be dosed with mineral acids of pH 2 to 3 for the purpose of inhibiting bacterial growth if the time of treatment is not too long. It is well known that yeast will grow satisfactorily in the pH range 4 to 5, and this is usually taken advantage of in the factory to prevent development of bacteria. In general, spore-forming bacteria are no problem because they, as most other bacteria, fail to grow in the slightly acid fermentor liquid. Most molds and other fungi also find fermentor conditions unfavorable for development and are easily destroyed by the steaming of lines and other equipment. Certain wild yeasts and fungi closely related to the yeasts, however, may be a menace because their optimum growth conditions coincide with those for cultivated yeast. Their development may even exceed that of the commercial yeast. *Monilia, Oidium,* and the so-called mycoderma can be troublesome if allowed to get a start. With adequate pure-culture equipment, careful control, and daily sterilization of plant lines, tanks, and fermentors, contamination with such organisms rarely occurs.

In the past, various proposals have been made for the elimination of foreign organisms in the fermentor by the application of antiseptics. Effront[26] acclimated yeast cultures to salicylic, picric, and hydrofluoric acids. He also worked with formaldehyde. Hayduck[46] added formaldehyde or formic acid to the feed wort to control infection and claimed that on dilution of the feed wort in the fermentor, the antiseptics were no longer in a concentration high enough to injure the growing yeast.

In a well-operated yeast plant, all vessels and lines with which yeast and nutrient wort come in contact are kept clean, with detergents where necessary, and are steamed daily or between runs. To facilitate steaming, fermentors, mash tubs, wort-holding tanks, and yeast-cream tanks should be enclosed. The pure-culture equipment should be capable of withstanding several pounds of steam

pressure in order that it and its charge of starting wort can be thoroughly sterilized before cooling and inoculation. For aeration, sterile air must be supplied to the pure-culture vessels and well filtered air, to the fermentors. Hot solutions of detergents under good pressure are useful for hosing out tanks and fermentors. Automatic spraying devices have been used to clean the interior of fermentors. Yeast separators must be taken apart daily and hand cleaned. Cloths from the yeast presses can be conveniently cleaned with detergents in the ordinary laundry wheel, and the final wash water should contain a suitable antiseptic. The cloth-drying room should be supplied with filtered air and the dried press cloths should not be allowed to become contaminated with mold spores.

Air-borne contamination, especially that due to mold spores, becomes an increasing problem after the yeast is passed through the separators. Good sanitation in the press room and the yeast cutting and packaging departments must be rigidly observed, because in these places, solid yeast is exposed to the air and many molds will grow readily on the surface of a yeast cake. These departments must have clean air, clean walls and floors. Daily washing of yeast mixing and extruding machines, trucks, etc., is in order. Spraying of walls and floors of press and cutting rooms with antiseptic solutions and the application of ultraviolet irradiation are also useful means for avoiding unwanted infections.

The importance of a well-equipped bacteriological laboratory for good plant sanitation does not need emphasis. Frequent checks for sterility in process water, feed worts and plant equipment, close inspection of the yeast in all stages of manufacture for contaminating organisms, and intensive investigation to find the source of trouble if contamination should occur are some of the indispensable laboratory functions.

## YEAST PROPAGATION AND HANDLING

It is probably true in the United States that most yeast manufacturers follow similar patterns in fermentation procedures and other operations. In the majority of cases, the important raw materials are the same; keen competition has led to the use of similar equipment and of the most economical methods for the production of good-quality bakers' yeast at the best yields obtainable. Each company, however, has its own particular methods for the growing and the handling of yeast until packaged, the details of

which are generally held confidential. The fermentation scheme and plant processes as outlined in the following will represent, therefore, what is generally known in the industry and will indicate some of the allowable variations and limitations of the art.

The initial step in bakers' yeast manufacture is the preparation by the laboratory of a pure culture of a selected strain of *S. cerevisiae.* When the laboratory culture has fully developed, it is transferred to the first vessel of the pure-culture department in the plant. The small tanks employed there, usually referred to as "kettles," have been cleaned previously, charged with special wort, sterilized, and cooled. The first kettle is usually small, only a few gallons in capacity, and is equipped with cooling coils and aeration tubes. The gravity of the wort is usually such that a number of hours elapse before the first kettle is fermented out. The second and larger kettle is then inoculated aseptically with the contents of the first kettle and this, in turn, allowed to ferment to a low gravity. One or more similar fermentation steps may follow through the use of still larger tanks, called "yeasting tanks" or "prefermentors," until enough pure-culture yeast has been built up to start the making of the seed-yeast proper. The yield of yeast on raw materials in these early stages is low, probably not over 20%. While molasses and salts may be used in the preliminary steps of seed making, the wort may also contain special nutrients to insure vigorous and well-nourished starting yeast for the seed fermentor. The temperature is carefully controlled throughout and may be at the same or at a lower level than in following fermentations.

At the final seed-yeast stage, more intense aeration is employed and, if good yields are to be obtained, the feeding-in of wort during growth is necessary. The Balling of the starting wort in the fermentor, in case a feed wort is used, would ordinarily be lower than that in the kettles. Nutrient salts, containing ammonium and phosphate radicals, are required with molasses worts and pH adjustment must frequently be made, usually by running in aqua ammonia.

Seed-yeast fermentation tanks may be 5,000 to 10,000 gal or larger, their size depending on the charge received from the yeasting tank and on the quantity of seed yeast desired. Seed-yeast yields will, of course, vary, but are several fold greater than those obtained in the earlier fermentation steps where but little aeration and no wort feeding prevailed.

Seed yeast is normally separated from the spent beer by centrifuges. Washing is accomplished by dilution of the yeast cream and reseparating as many times as is necessary to remove the beer. The finished yeast cream is either stored in tanks or pressed for future use. In any case, cold storage and freedom from contamination are necessary. The seed yeast may be used directly to produce trade yeast, or one or more intermediate propagations may be made. In the second case, better yields are obtained because the initial seed stages are the least economical in respect to yield of yeast from the sugar utilized.

The last fermentation, which produces yeast for the trade, is usually started in a dilute salts-molasses solution together with the seed yeast in the fermentor and is characterized by strong aeration and a wort feeding-in program designed to give high yields and good final yeast quality. Salts are added, mostly during the early part of the propagation, and aqua ammonia is supplied to furnish part of the needed nitrogen. Ammonia also assists in maintaining the pH at the desired level. The fermentation tanks are equipped with aeration equipment and means for cooling which may consist of water sprays on the exterior of the tanks (if they are of metal construction) or inside coils. The working capacity of the fermentors will probably range from 10,000 to 30,000 gal, or more. The total capacity will, of course, be greater as space is needed for foam and rise of the liquid during high aeration.

The yeast is removed from the beer, at the end of fermentation, by centrifuges, the resulting yeast cream being diluted and reseparated several times to free the yeast from the impurities and coloring matter of the beer. The net yield of trade yeast, based on all raw materials taken, will range from about 50% to over 90%, depending on yeast strain, quality of raw materials, and operating conditions. In calculating yields, the seed yeast is deducted from the total production and a moisture content of 70% in the pressed yeast is assumed.

The yeast cream, containing about 4 lb of yeast per gal, is normally stored in refrigerated tanks until it can be pressed. Plate and frame, or similar presses, lined with heavy cloth are used for removing the excess water in the yeast cream. The yeast cake is scraped from the presses into suitable trucks or chutes which, in turn, deliver the product to the cutting and packaging room. Bulk cake from the presses must not be allowed to stand for any length

of time, because even in a refrigerated room, it tends to heat spontaneously and may, in a few hours, become badly damaged. In the packaging room, the yeast cake is placed in mixers, adjusted to about 70% moisture, and small amounts of vegetable oils are added to facilitate cutting and to achieve firmness and good appearance of the finished cake. Special equipment is used to extrude the mixed yeast, usually in the form of a continuous solid bar. Other machines cut the bar in pieces of definite weight. These pieces are then fed into automatic wrapping machines from which the wrapped pieces are hand packaged or placed on racks for cooling. Whether boxed immediately or racked, the wrapped yeast must be thoroughly chilled to prevent spoilage.

For the handling of such liquids as yeast cream and prepared wort, lines constructed of copper, brass or stainless steel are advisable. Centrifugal pumps for circulation are preferred to reciprocating pumps as they are more sanitary and less likely to cause maintenance problems.

## GENERAL PLANT EQUIPMENT

Ideally, a yeast plant should be located close to raw material sources and should be able to sell all its output in the near-by territory. It should have an abundance of pure, cold water, cheap power, no waste-disposal problems, and the best type of sanitary equipment. Generally, however, plants have been set up in locations where transportation costs are not always low, where good water may not be cheap, and where the disposal of plant effluents is costly. The stream-polluting capacity of a large yeast plant is equivalent to that of a fair-sized city. Wooden fermenting tubs, rather than metal tanks, may sometimes be a necessity for economic reasons.

Practically, steel storage tanks for molasses, wooden tubs for mashing, and nonferrous lines and fittings, and tinned-copper fermentors are satisfactory. Adequate refrigeration is necessary for cooling yeast cream and stored packaged yeast. Other equipment needed includes wort filters, usually of the pressure type, such as the Sweetland or plate and frame filters. Centrifugal separators for yeast are a costly, but essential part of the plant.

For each pound of yeast produced 5 to 10 gal or more water may be used, a considerable portion of which is required for cooling during fermentation. Power is required for compressed air, refrigeration, the yeast separators and the cutting and packaging

equipment, for operating pumps, and for many other purposes. Considerable steam is needed for mashing, heating water, and sterilization. Low-pressure steam for processing may be derived from the exhaust of turbines, which are employed to drive generators or blowers. The problems of boiler capacity and current generation, or current purchase, will be different for each plant.

## AERATION

Aeration of the liquid medium during yeast growth is necessary for good yields. De Becze and Liebmann,[6] in an extensive review of the literature on aeration, estimated that with ordinary perforated aeration tubes in the fermentors, air consumption amounts to 0.1 to 0.3 cu ft per minute for each gallon of liquid, or, expressed in other terms, each pound of yeast made will have required 330 to 530 cu ft of air. They also stated that power for aeration represents 10 to 20% of the cost of yeast production. The same authors discussed at length the relative merits of perforated tubes and porous ceramic aeration devices. Greater resistance to air flow, greater maintenance cost and increased cleaning problems are probably some of the reasons why porous aerators are not popular in the United States. Stich[107] was one of the leading exponents of the porous-tube type of aerators and his inventions apparently met with some success in Europe in cases where both alcohol and yeast were produced simultaneously. The "fine aeration" obtainable with ceramic candles calls for less air volume but increased pressure, and, with it, less alcohol is evaporated and fair yields of yeast are said to be produced.

Equipment for producing compressed air may be of any conventional kind, reciprocating compressors, turbines or Root-type blowers. Orifice plates in the lines are convenient for controlling air quantities. The air should be filtered and cooled before entering the fermentors.

Numerous devices and procedures have been invented to supposedly improve aeration. One working method[66] calls for the introduction into the fermentor of a mechanically made foam of air in molasses which has been stabilized with small amounts of dextrin. For operating at a lower air pressure, Wessblad[113] continuously aerated a portion of the fermentor contents on the outside of the tank and then returned it to the main body of liquid. A Swedish patent[89] claimed improvement through mechanical agitation of

the liquid over and around an air inlet to the fermentor. Several methods[79,80] have been designed to break up large air bubbles and to delay their escape by means of propellers or agitators moving in the fermentor. Vogelbusch's patent[112] involved this principle and his equipment has been used with some success in Europe. Vogelbusch's agitator consisted in streamlined and perforated hollow blades supported by a central hollow shaft through which air was forced to the blades. Reduction in air pressure was claimed by reason of the high liquid velocity over the rapidly revolving elements and fine air bubbles were said to result through a shearing action of the liquid as the propellers rotated. The air holes in the blades were relatively large, about 2 mm in diameter.

Improvements over ordinary perforated tubes for the distribution of air in the fermentor have been sought by various inventors. Lockey[70] attempted to obtain fine air bubbles by forming a screw thread on the perforated tubes and then winding a wire in the cut grooves. Forcing air through short bundles of wire, or between closely packed flat strips of metal are other variations for producing small bubbles.

Rapid circulation of the fermentor contents by means of an outside pump, so that the total volume of fluid is removed from and returned to the tank in a few minutes, has been proposed as an alternative to ordinary aeration.[55] Variation in the volume of air introduced into the fermentor by means of an automatic control device during yeast growth was the subject of a patent to Rosenqvist.[90] Bratton[11] used a closed system to prevent loss of alcohol, volatile organic acids, and esters. Exhaust air and gases were pumped back into the fermentor, or into another fermentor, a small volume being allowed to escape as some fresh air was introduced as needed.

The use of lactic or acetic acid to reduce surface tension and promote fine aeration, especially with porous tube equipment, was patented by Braasch.[9]

The exact function of aeration in yeast growth has been the subject of considerable speculation. Reportedly fair yields have been obtained by mechanical agitation alone or by agitation with inert gases. However, oxygen has usually been introduced into the fermentor by some means or other to produce satisfactory crops of yeast. Agitation with air may be considered beneficial from several standpoints; it establishes an aerobic condition in the

nutrient solution, it removes excess carbon dioxide, and maintains uniform concentrations of both yeast and yeast nutrients throughout the solution.

Working with malt worts, Slator[102] concluded that in the initial stage of the fermentation, yeast growth is retarded by air, but that later oxygen is beneficial since it aids in the removal of carbon dioxide. Schultz, Atkin and Frey,[94] however, claimed that the fermentation of maltose and galactose was hastened in the early stages by the use of pure oxygen. Braasch and Braasch[10] found that excessive aeration could be harmful in removing too much carbon dioxide. They claimed that, in such cases, 1 to 3% of carbon dioxide should be contained in the air blown into the fermentor. Frey, Schultz, and Atkin[34] stated that oxygen depresses anaerobic fermentation by one half.

## FOAM CONTROL

One of the troublesome aspects of yeast manufacture is excessive foaming in the fermentors during aeration. Its control is an expensive item. Many kinds of animal and vegetable fats and oils, or products derived from them, have been employed to hold the fermentor foam within bounds. Experience has indicated that there is some correlation between an effective defoamer and the raw materials employed for mash preparation; usually only actual tests will reveal the most suitable and economical agent to be used under existing plant conditions. According to a German patent,[52] aliphatic alcohols containing eight or more carbon atoms will control foaming successfully. A patent to Steibelt[105] covers a mixture of equal parts of hexadecanol, tetradecanol and paraffin oil for defoaming.

Mechanical contrivances have been devised to destroy foam, or limit it, but apparently they havè not been too successful. Illustrative of endeavors in this direction is a patent of Hayduck[47] which describes an air jet within the fermentor that sucks in foam and then propels it against a baffle plate. This action is said to break down the foam into a liquid which either may be returned to the fermentor or removed from it. Automatic control of additions of a defoamer to the fermentor as needed has been attempted[30] through the use of a photoelectric cell and the necessary auxiliary equipment.

## YEAST SEPARATION

After the yeast is matured in the fermentor, the next operation is separation. Centrifuges of 5,000 gal per hour capacity, more or less, remove the yeast from the spent beer in the form of a cream. Since the liquid surrounding the yeast in the cream is mostly dark-colored beer, this must be removed by repeated dilution with water and reseparation until the cream is practically free from color and waste products.

Washing may be accomplished in several ways. Water can be added to the separators with yeast cream; the cream may be diluted with water in a wash tank; or cream and water may be mixed together in special nozzles which are placed between two or more banks of separators. A separate wash tank allows for much flexibility in operation, while a washing nozzle requires exact adjustment of flow of liquids and uninterrupted separator performance.

## YEAST CUTTING

The forming of pressed yeast into pieces of fixed shapes and weights for the trade is ordinarily referred to as "cutting" yeast. The customary weights are 1 lb for the bakeries and ½ lb and ½, 1, and 2 oz for the grocery trade. The weights of smaller pieces may vary somewhat from the figures given. Waxed paper for wrapping is customary except that the smallest pieces may be covered with metal foil. Both cutting and wrapping is accomplished by machines in most plants.

Wrapped and packaged yeast tends to lose a small amount of water when placed under refrigeration, the water condensing on the inside of the waxed wrapper in the form of droplets. These water droplets may slightly discolor the surface of the yeast cake and to overcome this defect, Farrel[32] patented a special wrapper which consisted of an inner layer of paper with high water-absorbing capacity covered by an outer layer relatively impervious to moisture. The two layers were cemented together by wax which could be softened sufficiently to seal the folded ends in the packaging machine. Lewton[68] attempted to coat yeast with a film of mineral or vegetable oil to seal the surface and prevent deterioration.

To improve the color and cutting characteristics of yeast,

Schultz and Frey patented[98,99] propylene and sorbitol laurates, and the laurates of polyhydric alcohols, such as glycol, glycerol, and inositol as additions to yeast prior to cutting. They also claimed patent coverage in yeast cutting[97] for lecithin and other emulsifying agents, like diglycol mono- and dioleates, ammonium oleate and ammonium linoleate, glycerol monostearates and diglycol mono- and distearates. Claims have been made[16] for enhanced baking strength in yeast through the incorporation at mixing time of such diverse substances as mucic acid, calcium acid phosphate, urea, papain, and oxidizing salts. Protection against mold infection in yeast through addition of ethyl, propyl, isopropyl, butyl or amyl alcohol has been also claimed.[14]

## ACTIVE DRY YEAST

Dried yeast for home baking, in the form of a mixture of cereal materials and dehydrated yeast cells, was used for many years in rural districts and other places where fresh compressed yeast was not readily available. Its activity was poor and the yeast cakes required an overnight soaking in water before use; the resulting bread was apt to be coarse and of inferior volume. In making the product, a paste of cereals and hops was prepared and, after being mixed with liquid or pressed yeast, was formed into small cakes and slowly air dried. Patents to Hill and Givens[49] described some of the practical methods followed in manufacturing dried yeast of this type.

Recently, so-called "active dry yeast" has replaced the cereal-yeast mixture for home baking and has found acceptance in commercial baking as well. The new product, consisting of only dry yeast cells and containing no filler, is able to ferment sugar vigorously after a few minutes soaking in warm water. It contains about 8% moisture, does not require refrigeration, and will remain active under normal atmospheric conditions for many months.

The older patent literature on active dry yeast contains widely varying, and frequently contradictory, claims on such subjects as fermentation procedures, conditions for drying, and additions to yeast before drying. Apparently, none of the processes disclosed were found to be commercially practicable.

Present manufacturing methods are held confidential and very little information on the subject of active dry yeast has been disclosed through recent publications. Shaver and Frey,[100] in 1933,

patented a process for preparing yeast for drying, according to which ordinary yeast is aftertreated in a molasses solution, containing assimilable organic nitrogen, for lowering its protein content below 50% and raising its glycogen content to 5% or more, both values being calculated on dry yeast solids.

Enright and Foote,[28] 1944, also subjected commercial yeast to a special conditioning fermentation prior to dehydration. According to their process, the yeast quantity was increased 40 to 50% in the final fermentation, but the activity was reduced somewhat. They stated, however, that yeast so treated could be dried without injury. The conditioning wort contained, in 234 gal of water, 17.66 lb sugar, 0.20 lb ammonium tartrate, 0.30 lb calcium monohydrogen phosphate and 40 lb compressed yeast. Strong aeration was employed and the finished yeast was considered satisfactory when its nitrogen had been reduced to about 6.5% (dry basis).

Auden and Eaglesfield[3] claimed improvement in the drying characteristics of yeast and greater stability in the finished product through the use of fruit pectin and the colloids obtained from the aqueous extract of flaxseed. Such substances, mixed with the yeast before drying, were said to prevent a too rapid dehydration and later to act as stabilizers of the residual water left in the dried yeast.

Yeast, suitably conditioned for drying, is pressed, comminuted by extrusion, and dried under controlled conditions in tunnel, continuous conveyor-type, or rotatory driers. The yeast from the drum driers is finished in the form of round pellets, but that produced in the other types of driers retains its original extruded form.

A few years before World War II, active dry yeast for home baking was successfully established. During the war, all of the nation's production was utilized by the armed forces. It was possible for the first time, because of the availability of dry yeast, to supply fresh bread to soldiers at or near the front lines in all foreign fields. After the war, the armed forces have continued to use dry yeast outside of the United States. The export of active dry yeast to Latin America and Pacific areas is steadily increasing. A new development of importance is the marketing of prepared mixes for rolls and bread, containing a separate enclosure of dry yeast. A number of commercial bakers have substituted dry yeast for compressed yeast successfully. The product offers distinct advantages over compressed yeast in regard to reduced costs of transportation

and refrigeration (which is necessary with compressed yeast). It also has the advantage of much longer life and can be stocked in considerable quantities.

Dry yeast for baking is sold in packages containing 1 to 300 lb. Fiber drums are frequently employed for shipment in the domestic market, sealed cans more often for export. For home baking and prepared bread mixes, the yeast is customarily packed in laminated paper or foil envelopes in fractional-ounce quantities.

Extended life of dry yeast is accomplished by vacuum or inert-gas packing. A patent to Atkin, Kirby, and Frey[2] claimed better preservation of the activity of dry yeast by elimination of oxygen through vacuum packing or by replacement of air with nitrogen or carbon monoxide. A recent investigation of the deleterious effect of air on active dry yeast was made by Oyaas, Johnson, Peterson, and Irvin.[81] The report of this study likewise indicated that dry yeast stability was improved through elimination of air by vacuum packing or by the substitution of nitrogen or carbon dioxide for air. Some improvement was found with only partial elimination of the air. The yeasts used in this investigation were from commercial packs made by several manufacturers for the armed forces.

## LABORATORY CONTROL

In addition to pure-culture maintenance and control of infection by microorganisms, the plant laboratory must make chemical analyses of raw materials and finished products. Besides the chemical and bacteriological departments, equipment and trained personnel for test baking of yeast is absolutely necessary. While the chemical analysis of yeast is important, its results cannot be substituted for information gained by observing the actual performance of yeast in bread dough.

In respect to raw materials, knowledge of their content of total solids, sugar, nitrogen, total ash,. phosphorus, alkaline earths, trace elements, and growth-stimulating factors is important, both from the standpoint of cost and of their action in the fermentor. Sugar may be determined by the polariscope or by copper reduction. The usual procedures are employed for invert sugar, before and after inversion of the sample, and for the estimation of such sugars as raffinose. The Kjeldahl method for total nitrogen is commonly used in the analysis of both raw materials and yeast. Methods for determining the ash and water content of raw materials and of yeast

will probably vary widely from plant to plant, as is usually the case with these two constants. Phosphorus can be conveniently estimated by one of the colorimetric methods. Microbiological and other standard tests can be applied in the assay for growth stimulants.

Reliable pH meters are essential in the laboratory as well as in the plant where constant checking of the acidity in the fermenting medium is necessary for proper control of yeast growth. And in both plant and laboratory, hydrometers, usually made with the Balling scale, are necessary instruments.

The finished yeast should be tested for moisture, acidity, nitrogen and phosphorus content. In some instances, vitamin assays are important. Knowledge of the ash, fat, carbohydrate fractions, and amino acid structure of yeast may also be desired. The laboratory will check the yeast microscopically for abnormalities, such as foreign organisms, dead yeast cells, and unusual budding conditions. Such inspection should be made during yeast growth and in the final stage of manufacture.

The baking laboratory should be able to produce all ordinary baked goods under rigidly controlled conditions to assure the yeast manufacturer that his product will be satisfactory for his customers. Certain daily control tests in the baking laboratory will be set up, the results of which, together with essential chemical analyses, should be available immediately to the manufacturing department. The final yeast will have been tested more than once by baking before it is shipped. Suitable exposure tests should also be devised to furnish information regarding the stability of finished yeast under possibly adverse conditions in the field.

The laboratory can give valuable assistance in improving methods for processing raw materials and developing better plant methods. Strictly speaking, these are functions of a research laboratory, but without some developmental program, either in the control laboratory or in a separate department, the modern yeast manufacturer will soon find that he is falling behind competition.

## VARIATIONS IN FERMENTATION PROCEDURES

The patent literature is replete with methods claimed to be improvements in working procedures for manufacturing compressed yeast. The following few references will indicate some of the modifications attempted for securing better yields, conserving fermentor space, improving yeast quality, etc.

During fermentation, Jacobsen[54] made adjustments in the rate of feed-wort additions to coincide with yeast budding, his theory being that nutrients should be supplied in greater amount to the yeast just before budding starts and that relatively less food should be given after the buds are formed. De Becze[5] assumed that for yeast of good quality, the feed wort should be supplied to the yeast according to its needs during the early stage of growth, but at a relatively lower rate in the latter period of propagation. Believing that yeast may be weakened by forced growth, Moskovits[75] proposed an aftertreatment which consisted in subjecting the yeast to low aeration in a solution rich in organic nitrogen followed by the final step of increased aeration and a higher sugar concentration in the wort. Klein[62] suggests that the characteristics of commercial yeast are determined partly by the quantities and types of substances employed in the last growth stage. He remarks that, for the nearly complete assimilation of carbon or nitrogen containing nutrients, one or the other must occur in excess, e.g., for the complete assimilation of available carbon, excess nitrogen must be present in the medium, and vice versa.

The best utilization of sugar is stated by Howells[51] to occur when, starting 2 hours after seeding, the rate of sugar feed is maintained at 1.26 times that supplied to the yeast in the preceding hour. He also recommends that the setting wort in the fermentor should be higher in assimilable nitrogen than the feed wort and states that the ratio of sugar to nitrogen should be 17.5 to 1 on the basis of the total wort employed.

Kirby and Frey[58] claimed improvement in yeast quality by the use of relatively large amounts or high-protein seed yeast, e.g., 30 to 60% of the total molasses. The seed yeast should have a protein content of over 50% (dry basis) and the final trade yeast less than 50%. They also claimed better stability in the pressed yeast by using at least 20% of high-protein seed yeast and limiting the supply of phosphate (as ammonium acid phosphate) to 1.25 to 2.0% of the molasses and the inorganic nitrogen (as ammonium sulfate) to 8%, so that the finished yeast will have less than 50% protein (dry basis).

Lutz and Irvin,[71] to save cooling water, power for aeration, and fermentor space, proposed the use of a small volume of setting wort to which a concentrated feed wort, together with cold water, was gradually added. The added cold water supplied a substantial

proportion of the necessary cooling and the lower initial liquid volume in the fermentor reduced back pressure in the aeration system. If high seeding were used at the start, it was claimed that the process could be modified by distributing the contents of the first fermentor among several other tanks and continuing the gradual additions of concentrated feed and water.

A reduction in seed yeast quantity and increased production per fermentor was claimed by Irvin and Mead[53] who used a two-stage propagation program. The first phase (6 hours) was started with low-seeding, high-concentration wort and light aeration; the second period (8 hours) called for increased dilution and more air. In the first stage 20 to 45% of the total nutrients were consumed. The process allowed for the distribution of yeast and solution among several fermentors at the termination of the initial step in the original tank.

## BAKERS' YEAST FROM SULFITE LIQUOR

Waste sulfite liquor from paper mills has been successfully used for the manufacture of bakers' yeast at favorable locations, as in Scandinavia and Canada, where there are large pulp mills and the usual raw materials for yeast making are less readily available. Heijkenskjöld was one of the principal workers in this field and has extensive patent coverage[48] on the manufacture of yeast from sulfite waste liquor. Because of the low sugar content and high nonsugar solids in sulfite liquor, radical changes in the customary procedures were necessary in its application to the production of bakers' yeast. According to Heijkenskjöld's process powdered limestone is added to the liquor for defecation and removal of sulfur dioxide. The liquor is then aerated and finally filtered. Alum and supplementary yeast nutrients, such as malt sprouts and ammonium salts, may be mixed with the liquor before filtration. Bleaching of the liquor with hypochlorites may also be employed. Yeast propagation is started in clarified liquor that has been especially enriched with such substances as molasses and compounds high in assimilable nitrogen. Later on, only sulfite liquor is taken as the nutrient solution. Aeration is employed throughout as usual. An important feature of the process is the continuous removal of part of the fermentor contents, separation of yeast from the beer, and return of the yeast to the fermentor. It was said that the yeast finally

removed from the fermentor may be subjected to an aftertreatment in molasses wort to improve odor, taste, and baking strength.

## MISCELLANEOUS DEVELOPMENTS

Improvements in yields of yeast by increased dilution, aeration, and continuous feeding of wort have been mentioned. Rainer[84] and Hayduck[42] demonstrated that a low concentration of sugar at all times was essential for the highest yields, with little loss of sugar due to alcoholic fermentation; Hayduck assumed that the little alcohol formed was assimilated by the yeast. Ransohoff[85] also believed that yeast would utilize alcohol under suitable conditions. As an alternative to the feeding-in procedure, Wroten[119] attempted to limit the available sugar by enzymic control of the rate of sugar formation from liquefied starch present in the nutrient solution. According to a process developed in Austria,[56] part of the wort was subjected to an alcoholic fermentation, the alcohol thus formed being later employed as a source of carbon for the yeast in the final stage of growth. In respect to alcohol utilization by yeast, the conclusion of Claassen[19] that no large amount of alcohol is assimilated as long as sugar is available is probably valid.

With the advent of the salts-molasses method for yeast growing, there arose the problem of controlling the excess acidity due to liberation of free mineral acids from ammonium salts. A solution of the problem, through neutralization with such substances as sodium carbonate, lime, and ammonia, was found by several independent groups of workers at about the same time and similar patents on the subject were granted in 1923 to Hayduck,[41,43] Kohman, Irvin and Cross,[64] Corby and Glasgow,[21] and Nilsson and Harrison.[77] Deleterious acidity in salts-molasses fermentations, originating from anions set free from ammonium salts, probably develops only when a combination of high dilution, strong aeration, and the feeding-in process exists. Neutralization with ammonia functions very well because most of the neutralizer is finally consumed by the yeast as a source of nitrogen.

An improvement, which has been given considerable attention, but which has not been universally adopted, is the continuous process for yeast manufacture. Though others[44,45,84] had worked on the problem, Sak[92] was one of the first to study it intensively. He drew off some of the fermentor contents and separated the yeast from it, replacing enough yeast, water, or nutrient solution to

maintain constant conditions in the fermentor. Daranyi,[24] working with high concentrations of both sugar and yeast, drew off one-sixth of the fermentor contents every 2 hours and subjected the withdrawn portions to further aeration in a separate tank to recover residual nutrients before separation. Strong wort was added to the fermentor to reestablish the original concentration. He claimed a uniform average of 8% yeast in the fermentor and nutrient levels several times greater than those customary. An improvement in continuous operation was developed by Meyer[73] who occasionally interrupted the molasses feed and substituted a grain-wort feed, prepared from malt or malt sprouts, for the purpose of periodically strengthening the yeast. A German patent[8] described a complicated system of fermenting vessels through which the yeast-growing medium was circulated and enriched at suitable points with fresh wort or other nutrients. Corby and Buhrig[20] claimed increased yields of yeast and greater plant capacity by a modification of the continuous process which included periodical withdrawals from the fermentor and the simultaneous additions of nutrients.

Several investigators have sought increased yields of yeast, or other benefits, by variations in temperature during propagation. Meyer and Chappe[74] claimed increased production by using an initial temperature of 86°F and then decreasing it to 75°F after about 4 hours. The temperature was maintained at the lower level until the end of the fermentation, or was allowed to rise to 86°F during the last 2 hours. Corby and Buhrig[20] also started propagation at a higher temperature (33°C), and finished at a decreased temperature (30°C). Fuchs[36] attempted to produce bakers' yeast from brewers' yeast by gradually acclimatizing the latter to 95°F through successive stages of propagation which started at the low temperature of 41°F.

Irradiation of yeast or yeast-nutrient solutions with ultraviolet light has been given some attention. Reinisch[86] claimed that such treatment of water and wort, before seeding with yeast, benefited the fermentation and also enhanced the vitamin content of the yeast. Improvement in the yield of yeast by exposure to electromagnetic wave lengths of 1.8 mm to 120 m was patented by Liebesny and Wertheim.[69] Reported[103] findings by Fernbach, Linder, and others have indicated beneficial results of various types of irradiation, improvement in pitching yeast (brewers') and the fermenting power of bottom yeast by light treatment and stimulation of yeast growth

by radium emanations being mentioned. Aberhalden,[1] however, reported a deleterious effect of ultraviolet light on yeast and Woodrow, Bailey, and Fulmer[118] showed that the shorter wave lengths of the ultraviolet portion of the spectrum produced toxic substances from wort ingredients, such as sugar and that such irradiated wort would not support yeast growth.

Pressed yeast that will not readily disperse in water and remains at the bottom of the vessel in the forms of discrete and visible particles is said to be "gritty." Flocculating or gritty yeast has been variously attributed to raw materials, fermentation conditions, and yeast strain. Unquestionably, some strains have an inherent tendency to flocculate which is absent in others. It has been found that a tendency to grittiness in a yeast strain can be overcome by periodical additions of the feed wort instead of the usual continuous feeding-in method.[60] While some earlier workers attributed grittiness to the newly developed salts-molasses worts, Koch[63] pointed out that it was a characteristic of the yeast strain and independent of the composition of the mash. Grittiness is usually absent in the early stages of yeast propagation and is evident only near the end of the manufacturing process. Change in the electric charge on the yeast cells has been considered as a possible explanation of the clumping phenomenon. Since the cells normally carry a negative charge, it has been suggested that late in the growth period some cells enter a resting stage, becoming positively charged and thus attract the negatively charged yeast to form clumps. As the pH of the solution in which the yeast is suspended is increased, a reversion of charge on the cells occurs and this fact also has been mentioned as a possible explanation of clumping.

In addition to interrupted feeding, the use of mostly organic nutrients has been considered as a possible remedy for grittiness. In practice, the selection of suitable strains has led to pressed yeast that is free from grittiness.

The literature on yeast growth factors is quite extensive and, while of only academic interest in many instances, the yeast manufacturer may profit by it. Corn steep liquor, malt sprouts, and cane molasses, for example, are richer in certain growth stimulants than beet molasses. Some of the beet molasses are more conducive to high yields than others irrespective of their sugar content. Prescott and Dunn[83] list inositol, pantothenic acid, biotin, thiamin, and beta-alanine as growth stimulants and mention that growth-promot-

ing complexes are obtainable from many natural sources, such as molasses, hydrolyzed proteins, and extracts of plants, yeast, malt, and malt sprouts. Hayduck,[40] working with cane sugar in a salts-sugar medium, found that his yields were strikingly decreased as the purity of the sugar increased. Williams[115] patented beta-alanine and related active compounds as growth promoters. Benefits derived from the combination of beta-alanine and aspartic acid were mentioned and the statement was made that the results found were partly a function of yeast strains. Beta-alanine hydrochloride, according to Williams, was effective in concentrations of as little as one part in 12,000,000 parts of medium. In another patent, Eakin and Williams[25] covered the use of calcium pantothenate, "biotic acid," and vitamin $B_6$ in yeast production. Combinations containing thallium, zinc, manganese, boron, iron, copper, and iodine were also claimed by them. Patent coverings granted to Schultz, Atkin, and Frey[93,95,96] included the use for yeast propagation of inositol, beta-alanine, thiamin, vitamin $B_6$ and a factor, $II_B$, derived from cane molasses and distillery slops: $II_B$, recovered by charcoal adsorption, was said to be effective in promoting the assimilation by yeast of the nitrogen of carbamide and, to a less extent, of ammonium salts. Vitamin $B_6$ was observed to overcome the inhibiting effect of thiamin on the Gebrüder-Mayer yeast strain. It has been claimed[111] that certain combinations of amino acids adversely influence yeast growth; tyrosine plus glycine was found to produce poor growth, while glycine alone, or in combination with phenylalanine, supported normal growth.

Fulmer and Husselmann[38] reported the formation of yeast stimulants by heating a synthetic medium under pressure, and stated, for example, that stimulants resulted from the heating of sugar solutions with phosphate and ammonium salts. Sherwood and Fulmer[101] found that the beneficial influence of ammonium chloride on yeast growth was apparent at lower temperatures, but that the salt appeared to be without effect at 40°C. In its active temperature range, ammonium chloride was said to increase both the yeast crop and the rate of cell development at the logarithmic growth stage, mathematical equations being formulated to show the relationship of temperature, normality of ammonium chloride, and rate of cell proliferation in both malt wort and synthetic media.

Richards[87] placed the optimum concentration of calcium sulfate for the best yeast growth at 0.0001 *M*, and stated that higher

values had an inhibitory action. He mentioned the findings of others which indicated that phosphates favorably stimulate fat formation in yeast. Thorne,[110] using synthetic media, observed that yeast strain 6479 at first assimilated ammonium phosphate in preference to amino acids. Later, relatively more organic nitrogen was said to be utilized, especially in the case of asparagine. Thorne mentioned that, during growth, the protein content in yeast is first relatively high, but is much lower at the termination of growth. The total excreted nitrogen was said to amount to 14%.

## BIBLIOGRAPHY

1. Abderhalden, E., *Fermentforschung*, **9**, 3071 (1927).
2. Atkin, L., G. W. Kirby, and C. N. Frey, Canadian Patent 411,325 (1943).
3. Auden, H. A., and P. Eaglesfield, U. S. Patent 2,111,201 (1938).
4. Balls, A. K., U. S. Patent 1,759,536 (1930).
5. Becze, G. de, U. S. Patent 2,199,772 (1940).
6. Becze, G. de, and A. J. Liebmann, *Ind. Eng. Chem.*, **36**, 882 (1944).
7. Bennet, W. G., and A. M. Peake, U. S. Patent 1,860,832 (1932).
8. Boye, A., German Patent 534,108 (1927).
9. Braasch, A., U. S. Patent 2,092,151 (1937).
10. Braasch, H., and A. Braasch, U. S. Patent 2,013,456 (1935).
11. Bratton, G. S., U. S. Patent 1,732,921 (1929).
12. Bratton, G. S., U. S. Patent 1,732,922 (1929).
13. Bratton, G. S., U. S. Patent 1,767,646 (1930).
14. Brougham, H., U. S. Patent 2,085,857 (1937).
15. Buhrig, W. H., and H. M. Harff, U. S. Patent 2,337,641 (1944).
16. Buhrig, W. H., A. Schultz and C. N. Frey, U. S. Patent 2,085,857 (1937).
17. Claassen, H., *Z. angew. Chem.*, **39**, 880 (1926).
18. Claassen, H., U. S. Patent 1,774,546 (1930).
19. Claassen, H., *Z. Spiritusind.*, **59**, 440 (1936).
20. Corby, R. L., and W. H. F. Buhrig, U. S. Patent 1,673,735 (1928).
21. Corby, R. L., and R. Glasgow, U. S. Patent 1,475,471 (1923).
22. Corby, R. L., F. M. Scales, and W. H. F. Buhrig, U. S. Patent 1,667,928 (1928).

23. Dahlberg, H. W., U. S. Patents 1,580,999 (1926); 1,581,000 (1926).
24. Daranyi, S. C., U. S. Patent 2,035,048 (1936).
25. Eakin, R. E., and R. J. Williams, U. S. Patent 2,322,287 (1943).
26. Effront, J., U. S. Patent 620,022 (1899).
27. Effront, J. I. A., U. S. Patent 1,176,528 (1916).
28. Enright, J. J., and H. E. Foote, U. S. Patent 2,346,671 (1944).
29. Epstein, A. K., U. S. Patent 1,858,488 (1932).
30. Everett, J., U. S. Patent 1,984,789 (1934).
31. Fabian, F. W., and L. J. Wickerham, *J. Bact.*, **31**, 31 (1936).
32. Farrel, R. A., U. S. Patent 2,441,477 (1948).
33. Fleischmann, H., U. S. Patent 102,387 (1870).
34. Frey, C. N., A. S. Schultz, and L. Atkin, *3rd Intern. Congr. Microbiol.*, **1939**, 743; *C. A.*, **34**, 5245 (1940).
35. Frey, C. N., A. S. Schultz, and A. P. Harrison, U. S. Patent 1,814,210 (1931).
36. Fuchs, E., U. S. Patent 2,072,748 (1937).
37. Fulmer, E. I., *Colloid Symposium Monograph*, **II**, 204 (1925).
38. Fulmer, E. I., and B. Huesselmann, *Iowa State Coll. J. Sci.*, **1**, 411 (1927).
39. Hamburger, R., and S. Kaesz, U. S. Patent 1,770,402 (1930).
40. Hayduck, F., U. S. Patent 1,449,102 (1923).
41. Hayduck, F., U. S. Patent 1,449,103 (1923).
42. Hayduck, F., U. S. Patent 1,449,105 (1923).
43. Hayduck, F., U. S. Patent 1,449,106 (1923).
44. Hayduck, F., U. S. Patent 1,449,107 (1923).
45. Hayduck, F., U. S. Patent 1,449,108 (1923).
46. Hayduck, F., U. S. Patent 1,449,112 (1923).
47. Hayduck, F., U. S. Patent 1,449,114 (1923).
48. Heijkenskjöld, G. O. W., U. S. Patents 1,680,043 (1928); 1,703,272 (1929); 1,881,557 (1932).
49. Hill, C. B., and M. H. Givens, U. S. Patents 1,641,676 and 1,641,677 (1927).
50. Hoffman, C., C. N. Frey, and F. M. Hildebrandt, U. S. Patent 1,687,561 (1928).
51. Howells, L. J., U. S. Patent 1,725,583 (1929).
52. I. G. Farbenindustrie A.-G., German Patent 602,087 (1934).
53. Irvin, R. R., and M. W. Mead, U. S. Patent 2,183,570 (1939).
54. Jacobsen, K. A., U. S. Patent 1,752,003 (1930).
55. Jansen, J., U. S. Patent 2,056,576 (1936).
56. Jellinek, E., Austrian Patent 130,438 (1929).
57. King, H. L., U. S. Patent 2,008,584 (1935).

58. Kirby, G. W., and C. N. Frey, U. S. Patents 2,029,572 and 2,029,592 (1936).
59. Kirov, A., and S. Leshchinskaya, *Brodil'naya Prom.*, **11**, No. 4, 22; *Chem. Zentr.*, **1936**, **I**, 1527; *C. A.*, **31**, 5936 (1937).
60. Kitzmeyer, E. L., U. S. Patent 2,097,292 (1937).
61. Kitzmeyer, E. L., U. S. Patent 2,150,329 (1939).
62. Klein, E., U. S. Patent 1,722,803 (1929).
63. Koch, R., *Wochschr. Brau.*, **45**, 187 (1928).
64. Kohman, H. A., R. R. Irvin, and R. J. Cross, U. S. Patent 1,475,494 (1923).
65. Kvasnikov, E., *Spirtovodochnaya Prom.*, **14**, No. 2, 22 (1937); *Chem. Zentr., 1937,* **II**, 2084; *C. A.*, **33**, 4736 (1939).
66. Lande, B. L. M. van der, and E. van Thiel, U. S. Patent 2,119,188 (1938).
67. Levy, S., U. S. Patent, reissue 10,341 (1883).
68. Lewton, L. O., U. S. Patent 1,597,450 (1926).
69. Liebesny, P., and H. Wertheim, U. S. Patents 2,107,830 (1938); 2,196,361 (1940).
70. Lockey, J., U. S. Patents 2,104,330 (1938); 2,176,558 (1939).
71. Lutz, S. L., and R. R. Irvin, U. S. Patent 2,084,736 (1937).
72. Mead, M. W., U. S. Patent 2,075,127 (1937).
73. Meyer, E. A., U. S. Patent 1,724,952 (1929).
74. Meyer, E. A., and P. W. Chappe, U. S. Patent 2,304,471 (1942).
75. Moskovits, M., U. S. Patent 1,962,831 (1934).
76. Neuman, B., A. Binz, and F. Hayduck, *Ergänzwerk zu Muspratts Enzyklopädischem Handbuch der technischen Chemie,* **4**, (1915).
77. Nilsson, M., and N. S. Harrison, U. S. Patent 1,449,127 (1923).
78. Norddeutsche Hefeindustrie A.-G., German Patent 590,209 (1933).
79. Norddeutsche Hefeindustrie A.-G., British Patent 423,331 (1935).
80. N. V. Industrieele Maatschappij v. h. Noury & van der Lande, French Patent 766,505 (1934).
81. Oyaas, J., M. J. Johnson, W. H. Peterson, and R. Irvin, *Ind. Eng. Chem.*, **40**, 280 (1948).
82. Pollak, A., U. S. Patent 1,558,627 (1925).
83. Prescott, S. C., and C. G. Dunn, *Industrial Microbiology*, 2nd ed., New York, McGraw-Hill, 1949.
84. Rainer, J., German Patent 10,135 (1879).
85. Ransohoff, F., U. S. Patent 1,722,858 (1929).
86. Reinisch, E., Austrian Patent 121,078 (1930).

87. Richards, O. W., *Ann. Report Soc. Chem. Ind. Prog. Appld. Chem.*, 505 (1925).
88. Rogers, D., and M. N. Mickelson, *Ind. Eng. Chem.*, **40**, 527 (1948).
89. Rosenqvist, S. O., Swedish Patent 88,558 (1937).
90. Rosenqvist, S. O., U. S. Patent 2,281,457 (1942).
91. Roth, G., U. S. Patent 1,170,110 (1916).
92. Sak, S., U. S. Patent 1,884,272 (1932); English Patent 308,324 (1929).
93. Schultz, A. S., L. Atkin, and C. N. Frey, U. S. Patent 2,285,465 (1942).
94. Schultz, A. S., L. Atkin, and C. N. Frey, U. S. Patent 2,311,418 (1943).
95. Schultz, A. S., L. Atkin, and C. N. Frey, U. S. Patent 2,322,320 (1943).
96. Schultz, A. S., L. Atkin, and C. N. Frey, U. S. Patent 2,333,955 (1943).
97. Schultz, A. S., and C. N. Frey, U. S. Patent 2,136,399 (1938).
98. Schultz, A. S., and C. N. Frey, U. S. Patent 2,223,464 (1940).
99. Schultz, A. S., and C. N. Frey, U. S. Patent 2,223,465 (1940).
100. Shaver, A., and C. N. Frey, U. S. Patent 1,910,265 (1933).
101. Sherwood, F. F., and E. I. Fulmer, *J. Phys. Chem.*, **30**, 738 (1926).
102. Slator, A., *J. Chem. Soc.*, **119**, 115 (1921).
103. Society of Chemical Industry, *Ann. Report Prog. Appld. Chem.*, 435, 481, 496 (1924).
104. Stagner, B. A., U. S. Patents 1,425,065 (1922); 1,457,319 (1923); 1,457,320 (1923).
105. Steibelt, W., U. S. Patent, 2,085,709 (1937).
106. Steinacker, P., U. S. Patent 2,187,990 (1940).
107. Stich, E., German Patent 567,518 (1927); U. S. Patent 1,792,450 (1931).
108. Terry G., U. S. Patent 88,590 (1869).
109. Thompson, J. W., and J. W. Hinchley, U. S. Patent 1,933,830 (1930).
110. Thorne, R. S. W., *J. Inst. Brewing*, **51**, 6 (1945).
111. Thorne, R. S. W., *J. Inst. Brewing*, **52**, 15 (1946).
111a. U. S. Dept. Commerce, Bureau of Census, Census of Manufacturers: 1947, Product Suppl., 18, 1950.
112. Vogelbusch, W., U. S. Patent 2,121,458 (1938).
113. Wessblad, K. A., U. S. Patent 2,118,370 (1938).
114. Wiedring, A. E., Red Star Yeast and Products, Milwaukee, Wisc., Private communication.

115. Williams, R. J., U. S. Patent 2,147,762 (1939).
116. Wohl, A., U. S. Patent 1,449,134 (1923).
117. Wohl, A., *Chem. Z.*, **52**, 202 (1928).
118. Woodrow, J. W., A. C. Bailey, and E. I. Fulmer, *Plant Physiol.*, **2**, No. 2, 171 (1927).
119. Wroten, J., U. S. Patent 2,106,784 (1938).

CHAPTER 10

# FOOD AND FEED YEAST

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## HISTORY AND DEVELOPMENT OF THE INDUSTRY

Medicinal uses of yeast were reported[15] to have been known to Hippocrates, to Pliny the Elder, and to others on through to modern times. While many such uses and observations could conceivably have been more or less connected with the treatment of nutritional deficiencies, it is difficult to determine when man first attributed value as a food to this protein- and vitamin-rich product. Whether the nutritional worth was recognized or not, it certainly entered the diets of many native groups in the form of fermented foods and beverages. Our present concepts, soundly based on careful chemical analysis, follow Delbruck's studies on the nutrition of yeast dating from 1875.[28] He recognized the feeding value of yeast cells and is said[17] to have strongly advocated recovery and use of waste yeast through the 1890's. Völtz and Baudrexel[45] fed yeast to human subjects experimentally and reported nutritional advantages in 1911. When World War I developed critical shortages of food and feed proteins, Delbruck, Hayduck, Wohl, and their coworkers, at the Institut für Gärungsgewerbe in Berlin, carried through a research program with the expectation of providing large-volume production

of yeast food and with results which radically changed the methods and substrates for growing primary type yeasts.

These German workers developed a process based on an earlier laboratory study of Hayduck and Hennenberg[9] for using the newly available and cheap sources of synthetic ammonia to replace the organic nitrogen previously used as a nutrient for yeast. At about the same time, they[8] substituted beet molasses for the more costly and scarce grains as a source of carbohydrates for yeast growth and energy. Commercial exploitation of the new process was rapidly expanded under the wartime economy, but unfortunately for the newly built yeast plants, a critical shortage of beet sugar and molasses developed in 1916 and forced shutdowns, which prevented full realization of the potentialities of these important advances until after the close of the war.

The molasses-ammonia process, later adopted by the yeast industry elsewhere and with various innovations and improvements to fit particular production requirements, remains one of the principal methods and sources of such primary-type yeast production as may enter the food- and feed-yeast fields. A modification of particular interest in the bakers' yeast field was developed by Heijkenskjöld[20] in Sweden and Finland around 1925 to 1928 and involved processing spent sulfite liquor to permit utilization of the hexose wood sugars for growth of *Saccharomyces* yeast. Economics of this bakers' yeast process have apparently not been such as to permit it to become an important source of yeast for direct human and animal consumption.

In 1934, as part of its Four Year Plan, the German government set up the Department for Economic Construction, the research program of which included extensive study of yeast production from wood sugar hydrolyzates and from spent sulfite liquor under the general direction of Schwabe.[38] Much of the fundamental research was again carried out at the Institut für Gärungsgewerbe but by a new generation of fermentation biochemists, under the leadership of Professor Fink and including Lechner and Just among the principal coworkers. Credit is due these men for significant advances in the basic knowledge and fundamental technology permitting highly efficient conversion of wood sugars and waste carbohydrates in economical production of food and feed yeast.

They based their work[35] on the original World War I studies of Delbruck and developed certain of his cultures of wild yeast to

the point where yeast-cell substance of exceptional quality could be grown in plant-scale equipment. Rapid growth rates and high degree of efficiency were achieved in conversion of hexose and pentose sugars, acetic acid, and a variety of other available carbon compounds to yeast-cell substances. They worked with the yeast, *Torulopsis utilis.* With characteristic German thoroughness, this research group studied and published extensively on torula-yeast production problems. Subjects studied ranged from composition of the spent sulfite liquor substrate to the final assay and evaluation of the dry yeast product. More than ninety papers by these authors are listed in the 1937-1946 Decennial Index of Chemical Abstracts, all of which are immediately pertinent to this extended research program.

Commercial exploitation of this new food- and feed-yeast process underwent rapid development with the onset of World War II in 1939. Following initial pilot-plant-scale development in co-operation with the German yeast and wood-sugar industries, at least three different new production systems reached the commercial stage in Germany. One was sponsored by the pulp and paper concern, Zellstoffabrik Waldhof and utilized a mechanical aeration system patented by Claus.[6] A second process was developed by the I. G. Farbenindustrie and utilized the aeration system of Scholler and Seidel.[37] A third process based on the earlier development of Vogelbusch[44] was adopted by an association of German yeast manufacturers prior to 1940 and used in several torula-yeast plants. Radically different methods of achieving such degrees of aeration characterized the three processes and all were aimed at elimination of the foam problem by mechanical means rather than by use of surface-active defoaming agents, such as the widely used lard and sulfonated oils of the bakers' yeast industry.

The onset of World War II also stimulated interest in yeast process development in countries outside Germany.

Thaysen,[43] at the Teddington Research Station in England, studied methods for production of improved cultures of *T. utilis* in molasses. Treatment of the yeast with camphor led to isolation of two special strains, one (var. *thermophilia)* being suitable for use in the tropics because of tolerance to higher than usual yeast-production temperatures and the other (var. *major)* being characterized by unusually large and easily centrifuged yeast cells. A

plant has been erected in Jamaica and is reported to have been in production since 1946.[2]

Similarly in Sweden, intensive study of process development was started in 1939, for a portion of which Rosenqvist gave an excellent description.[30,31] His process utilizes a "fine" aeration system similar in basic objectives to those in Germany and was developed especially for use on spent sulfite liquor substrates. A plant of this type is understood to produce bakers' yeast at Norrkoping, Sweden. However, no published record has been found of commercialization of that process in the food and feed yeast fields, and apparently production of such yeast in Sweden has been largely confined to utilization of maximum amounts of secondary type yeast available as an excess from the production of alcohol or from brewing operations. Evidence of continuing active interest in that country is gained in such recent publications on fundamental studies as those of Sperber[40] and Brahmer.[1]

Food yeast developments in Russia are not well known, but interest in the problem has been active from the early 1930's.[17,18] Acquisition of going plants in Russia's sphere of Germany could be expected to bring plant design and operating know-how up to date in the U.S.S.R.

In the United States, prior to 1942 the production of yeast for foods and for animal feeds was largely confined to the higher-grade specialty products of established baking-yeast manufacturers and to limited recovery and marketing of waste yeast by the brewing industry. Research and development on new processes, leading to establishment of a distinct food- and feed-yeast industry, has been increasingly active since 1943. Wartime shortages of high-grade protein feedstuffs served, to some extent, as an incentive for such process development, but were generally recognized to be of short-term duration. In this respect, United States developments differ from those of Germany and England. Most active work in this country seems to have been carried out for the purpose of finding long-term uses and outlets for waste carbohydrates of the fruit, vegetable, dairy, and forest industries. Alleviation of stream pollution by wastes of these types has been an important incentive. While we do have an extensive literature accruing from some of this research, much remains unpublished and may be expected to continue in that category because of the highly competitive future outlook.

In the more than 50 years since yeast was first produced as a

large-scale food and feed substance, a distinct industry has been developed. The recovery and use of waste yeasts, especially from brewing operations, has been highly developed and production has been maintained for years in volumes well up in the thousands of tons annually in the United States. Brewers' yeast retains an appreciable share of the market and, because of the low capital costs involved, probably will continue to do so as long as its strictly limited production capacity will meet the demand. In the years immediately following World War II, the primary grown yeasts based on cheap sources of carbohydrates began entering the market in expanding volume. Food- and feed-yeast plants built during the war in Germany and Switzerland are in postwar operation and their production is being supplemented by newly built plants in Finland, South Africa, Jamaica, Formosa (Taiwan) and the United States. Other plants are understood to be under consideration on a world-wide basis but especially in the carbohydrate-producing, protein-famine areas of the Far East.

## SOURCES OF FOOD AND FEED YEAST

Yeast can be and is produced from a wide variety of raw materials and by many variations of the growing process. It seems useful to attempt some classification of the principal sources of yeast known to have entered human food and animal feed markets in the United States and Germany, together with such other proposed sources as have been seriously studied on a pilot-plant or larger scale:

| | |
|---|---|
| Secondary Yeasts (Yeasts recovered as a by-product from alcohol fermentation) | —Brewers' Yeast<br>—Debittered Brewers' Yeast<br>—Molasses Distillers' Yeast<br>—Grain Distillers' Yeast (usually sold with dried grains and solubles) |
| Primary Yeasts (Processes designed to produce yeast alone with ultimate efficiency) | —Molasses Yeast grown on:<br>1. Cane molasses<br>2. Beet molasses<br>3. Corn sugar "hydrol"<br>—Refermented Yeasts (Secondary yeast, usually brewers' regrown for a generation or more in molasses)<br>—Spent Sulfite Liquor Yeast<br>—Wood Hydrolyzate Yeast<br>—Yeast from Agricultural Wastes |

Considered from the standpoint of volume actually sold prior to 1950, the secondary-type yeasts have been the most important source of yeast reaching the United States food and feed market. Their production is primarily a matter of economical methods for collecting and drying yeast and may involve the use of central drying plants when smaller breweries are concerned. The equipment required has been adopted more or less universally and the processing requires no detailed description here. The place of brewers' yeast in the nutrition field has been covered in an excellent review by Prouty.[27]

Aside from the technical advantages of controlling production for particular qualities of yeast, the importance of other sources of yeast of the primary type lies in ability to supply expanding markets over and above the limited production of the brewing and distilling industries. Brewers' yeast production has been variously estimated at 10,000 to 15,000 tons maximum annually in the United States on a potential not to exceed 25,000 tons. In contrast, primary-type dry yeast in volumes exceeding 100,000 tons annually could be supplied from processing of the carbohydrates readily available from either molasses or spent sulfite liquor and potentially also from wood hydrolyzates and agricultural residues.

The subject matter for the following discussion is, therefore, narrowed to certain, relatively new yeast-production processes, specifically designed for economical production of primary yeasts for the food and animal-feed markets and using low-cost sources of carbohydrates. In turn, the position of these new processes in the industry and their future importance depend primarily on the degree to which the market for food and feed yeasts develops over and above the supply of waste secondary-type yeast.

## RAW MATERIALS

Carbon compounds that can be utilized for growth and energy by the various yeasts are diverse and numerous.[12] Most important commercially are natural by-product sources of the hexose and pentose sugars and of the more or less related compounds, such as acetic acid which may be directly or indirectly derived from them.

### Grain

The carbohydrates of grain can be used for food- and feed-yeast production but, under normal conditions, are apt to be ruled out in

competitive production because of relatively high costs. In times or areas of overproduction of grain and again in times of acute protein shortage or yeast demand, these agricultural staples will be given serious consideration and could find extended use.

### Molasses

The residual mother liquors or molasses from the manufacture of cane, beet, and corn sugars, all originally classified as waste products, have enjoyed the favor of bakers' and some primary-type yeast manufacturers. Especially has this been true of beet molasses because of its widespread availability, low cost, high content of mineral nutrients, and the comparative ease with which a color-free yeast product could be produced from it. Adoption of the ion-exchange process for recovery of more sugar could reduce the amount of beet molasses entering the market. The blackstrap molasses of the cane-sugar industry is also used but, with this product, a greater degree of clarification is usually required. Hydrol from corn-sugar manufacture has been of increasing interest in this country in recent years. Table 30 lists representative basic analyses of these various types of molasses.

### Spent Sulfite Liquor

Spent sulfite liquor is the waste product of the sulfite-pulping process in the paper industry. It is frequently designated by the synonyms S.S.L., sulfite waste liquor and sulfite lye. Spent sulfite liquor has become an important source of carbohydrates with a large potential for yeast production. Approximately 400 lb of sugar, or the equivalent in the form of carbon compounds utilized by yeast, is contained in the waste liquor drained from each ton of pulp produced. With sulfite-pulp manufacture averaging more than 2,500,000 tons annually in the 10-year period 1939-1949,[47] some 500,000 tons of sugar were wasted in this spent liquor each year in the United States.

The first feed-yeast plant to use this raw material in the United States started production in 1948, with an initial design capacity of 4.5 tons of dry yeast daily. After 3 years of operation and continued process development, this plant at Rhinelander, Wisconsin, is considered technically successful in using the spent-liquor substrate. The earlier wartime utilization of spent sulfite liquor for dry-yeast production in Germany is confirmed and,

TABLE 30. REPRESENTATIVE ANALYSES OF VARIOUS TYPES OF MOLASSES

(As-received basis)

| | *U. S. Beet Molasses*[a] | | *Cane Blackstrap*[b] | | | *Cane High-Test Molasses* | | | *Corn Hydrol*[c] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Min. | Max. | Min. | Max. | Ave. | Min. | Max. | Ave. | Min. | Max. | Ave. |
| Specific gravity | 1.31 | 1.46 | 1.39 | 1.44 | 1.42 | 1.42 | 1.45 | 1.43 | — | — | — |
| pH (2:1 dilution) | 6.7 | 8.8 | 4.5 | 6.0 | 5.5 | — | — | 6.0 | 4.0 | 4.5 | 4.3 |
| Moisture, % | 30.0 | 33.8 | 16 | 23 | 19 | 14 | 19 | 17 | 26.4 | 27.4 | 26.9 |
| Dry matter, % | 66.2 | 70.0 | 77 | 84 | 81 | 81 | 86 | 83 | 73.6 | 72.6 | 73.1 |
| Total inverted sugar as glucose, % | 43.5 | 50.2 | 52 | 65 | 56 | 72 | 79 | 75 | 52 | 55 | 54.1 |
| Total nitrogen, % | 1.23 | 2.05 | 0.4 | 1.5 | 0.7 | 0.07 | 0.20 | 0.10 | 0.05 | 0.07 | 0.06 |
| Ash, % | 8.46 | 10.50 | 7 | 11 | 9 | 2 | 3 | 2.25 | 6.2 | 7.2 | 6.6 |
| Phosphorus, % | 0.005 | 0.012 | 0.6 | 2.0 | 0.9 | 0.2 | 0.6 | 0.3 | — | — | — |
| Calcium, % | — | — | 0.1 | 1.1 | 0.5 | 0.03 | 0.30 | 0.10 | — | — | — |
| Magnesium, % | — | — | 0.03 | 0.10 | 0.07 | 0.01 | 0.03 | 0.02 | — | — | — |
| Potassium, % | — | — | 2.6 | 5.0 | 3.6 | 0.7 | 1.4 | 0.9 | — | — | — |
| U. S. Production as tons of glucose for year 1947 | Data incomplete | | | 850,000 | | Little, if any, available during 1947-1950 | | | Data incomplete | | |

[a] Data from paper by P. N. Agarwal, K. Singh, P. S. King, and W. H. Peterson, *Arch. Biochem.*, **14**, 105 (1947).

[b] Data courtesy of F. W. Zerban, New York Sugar Trade Laboratory.

[c] Data courtesy of R. C. Wagner, Clinton Industries.

depending on yeast market developments, there is a possibility of considerable expansion of production in future years. Considered from the standpoint of producing yeast from this liquor as a method of waste disposal, the cost of sugar from spent sulfite liquor lies chiefly in the expense of collecting and preparing the liquor for yeast growth and as such, the substrate merits somewhat more discussion than would otherwise be assigned to a source of carbohydrates newly entering the field and as yet not fully evaluated.

The variation in concentration of the waste liquor available from different mills ranges from 60 g to 150 g of solids per l. The approximate composition shown in Table 31 varies somewhat with the kind of wood used and the method and degree of cooking in the pulping process. Hardwoods have a higher proportion of sugars, running up to 3% in liquors of similar dilution. The kinds of sugar are also a function of the wood used. Softwoods yield a product with total sugar of about 75% hexose, largely mannose. Liquors from hardwoods, such as beech and poplar, may have as much as 70% pentose sugars, mostly in the form of xylose. Hardwood liquors are also characterized by higher content of acetic acid.

TABLE 31. COMPOSITION OF SPENT SULFITE LIQUORS FROM SPRUCE WOOD[a]

| | Grams per liter | |
|---|---|---|
| Neutralized solids | | 100 |
| Total sugar (as glucose) | | 15 - 22 |
| Hexose | 11 - 16 | |
| Pentose | 4 - 6 | |
| Volatile acids (as acetic) | | 2 - 5 |
| Sulfur (all forms as $SO_2$) | | 8 - 10 |
| Total inorganic (free $SO_2$) | 0.5 - 2.5 | |
| Organic (loosely combined $SO_2$) | 3.0 - 5.0 | |
| Lignin (as lignosulfonate) | | 50 - 65 |
| Calcium | | 7 - 10 |
| Miscellaneous compounds, solvents, aldehydes, uronic acids, resins, etc. | | 2 - 5 |
| pH 1.5 - 3.0 | | |

[a] Variation of composition in liquors of the same solid concentration.

With very few exceptions, these spent liquors require pretreatment before use as a medium for yeast growth. Such pretreatment is primarily concerned with removal of excessive amounts of sulfur dioxide. For aerobic yeast growth, less attention to other toxic or inhibitive agents is needed although appreciable amounts of com-

pounds known to have deleterious effects on growth of microorganisms or blocking effects on enzyme systems are known to be present. The standard European process,[33,39] generally adopted in Germany for pretreatment of the liquor, is based on lime neutralization and usually involves aeration of the hot liquor just before the liming step. Close control of pH and subsequent clarification by settling for 12 hours or more, with or without phosphating, precede cooling and final use of the product. In the United States, steam stripping has been favored for removal of the sulfur dioxide.

## Wood Hydrolyzate

The sugar derived from waste wood by the Scholler and the Bergius processes and by their principal modifications have been widely advocated as a source of fermentation carbohydrates. Commercial plants have been built and were operated extensively in Germany and Switzerland before and during World War II.[33] A modified Scholler process, developed by the United States Forest Products Laboratory, was operated commercially in conjunction with an alcohol plant for a brief postwar period at Springfield, Oregon.[19] However, high capital charges, combined with lack of profitable use for the chief by-product, lignin, have prevented economical production of sugar and such plants have, in most cases, operated only under government subsidy. Even so, the huge potential supply of fermentable carbohydrates from such sources can hardly be ignored in view of the active research and development programs in progress and also when considering the large volume of excellent-quality yeast actually produced from this source in Germany during World War II.

## Agricultural Wastes

Much study has been devoted to the utilization of vegetable, fruit, and dairy wastes as a source of carbohydrates for yeast production.

Whey has been extensively studied in Germany and a newly constructed plant is operating for production of high-grade specialty yeast products from this source on a commercial scale in the United States. Carbohydrates from whey can be expected to be relatively expensive due to the cost of collection from scattered small sources of supply. However, high cost of the sugar could be partially offset

by supplementary content of phosphorus and other yeast nutrients in whey.

Potatoes, especially cull and surplus stocks, have potential value as a source of sugar for yeast growth. At least one plant was reported to have used potatoes in Germany.

Citrus peel hydrolyzates as substrates for yeast growth are being studied on a plant scale in Florida and Texas. No plants for commercial production are known.

Fruit wastes as a source of carbon compounds for yeast growth have been studied by the Western Regional Research Laboratory.[11] Pear wastes have been used for pilot-scale yeast production over a period of several years at Olympia, Washington.

In general, it may be concluded that agricultural wastes may serve as excellent substrates for yeast growth but are handicapped in many instances by the costs of collection and of pretreatment and by the unfavorable factor of seasonable supply which does not allow ready amortization of heavy capital investment.

## FERMENTATION MECHANISMS

The complex reactions by which sugars, nitrogen, phosphorus, potassium, and minor trace elements may be converted by yeast to new cell substance are not as well understood as are those involved in the mechanism of alcoholic fermentation (see Chapter 2). The fundamental studies of Effront,[10] Claassen,[5] Brahmer,[1] Fink,[13] and Sperber[40] do give us a general picture which seems to be agreed on by most authorities. The known course of reactions has been especially well described by Schmidt,[35] who shows that the sugars first undergo a phosphorylation and splitting to three-carbon compounds and then to two-carbon compounds, ethyl alcohol, acetic acid, and acetaldehyde. These reactions are summarized very briefly:

I. $C_6$–Stage: $C_6H_{12}O_6 + 2H_3PO_4 \longrightarrow C_6H_{10}O_6(PO_3H_2)_2 + H_2O$
Sugar diphosphate

II. $C_3$–Stage: $C_6H_{10}O_6(PO_3H_2)_2 \longrightarrow 2C_3H_5O_3 \cdot PO_3H_2$
Phosphoglyceraldehyde

$C_3H_5O_3 \cdot PO_3H_2 + O \longrightarrow CH_3CO \cdot COOH$
Pyruvic acid

III. $C_2$–Stage: $CH_3CO \cdot COOH \longrightarrow CH_3CHO + CO_2$
Acetaldehyde

For synthesis of new carbohydrate cell substance, these reactions are thought to be reversed. Fink and Lechner[14] have clearly shown that the two-carbon compounds, acetic acid, ethyl alcohol, and

acetaldehyde, are each suitable as a sole source of carbon for complete yeast growth and thus, amino acids, fats, enzymes and other carbon-containing cellular components can also be synthesized from these original $C_2$ compounds.

This brief summary leaves much to the imagination. We know that the nitrogenous fraction of the yeast cell is largely composed of enzyme protein and enzymologists recognize hundreds of separate enzyme entities, each with its individual capacity for bringing about one or more of the multitude of anabolic and catabolic reactions incident to life and death of the yeast cell.

Large amounts of energy from the carbohydrate food in the substrate are consumed in the synthetic processes and both theoretical and practical questions arise as to how much yeast can be produced from a given amount of carbon compound. Fink, Sperber and Brahmer in their publications have shown that of each 100 lb of glucose used by yeast, something over 49 lb but less than 59 lb of dry yeast were produced under ideal conditions. The exact amount between these limits remains to be agreed on.

Interpretation of the results of commercial yeast production is sometimes confusing on this point, since it is not uncommon to report the dry-yeast yield on the basis of sugar content with figures running to 65 or 70% from some substrates. Such media invariably can be found to contain more or less nonsugar carbon compounds, such as acetic acid, which may also be utilized by the yeast.

## CULTURES AND THEIR MAINTENANCE

Several varieties of yeast and yeastlike organisms have been advanced as suitable for use as food and feed material. More are being proposed as our knowledge of the subject expands. Most have desirable growth characteristics. However, a good many of these various organisms have received rather sketchy study from the standpoint of their desirability as a food and feed substance. As such, the number of organisms which we can name as known to be suitable for use in this field is not so large as it is likely to become in future years.

### Saccharomyces cerevisiae

The widely studied true yeast, *Saccharomyces cerevisiae,* has long been accepted in the industrial field and practically all secondary yeasts entering the food and feed market are obtained by

recovery of this organism. Those primary grown yeasts obtained by regrowth of secondary yeasts also derive largely from the same source. A disadvantage of the *Saccharomyces* yeasts lies in the tendency to utilize only the more expensive types of substrates for growth.

### Torulopsis utilis (Candida utilis)

Other organisms began to receive attention at the time of Delbruck's studies in 1915, but his cultures were not fully evaluated until the investigations of Fink and Lechner disclosed the full significance of the pentose-utilizing properties of *Torulopsis utilis*. *(Author's Note.* The generic classification of *Torulopsis utilis* caused considerable confusion since the isolation and study of the "Mineral Yeast of Hayduck and Haehn" in 1922. Lodder's 1952 taxonomic study "The Yeasts" places the organism in the genus Candida "because of evidence for formation of a pseudomycelium under anaerobic conditions." Use of the name *Candida* is being investigated by a committee appointed by the Seventh International Botanical Congress. In the meantime, it seems best to retain the industrial usage of *Torulopsis utilis* and the trade name "torula yeast" until official action settles the question.) This organism has since been the subject of much attention by nutritionists, microbiologists, and fermentation biochemists. Dry torula yeast has been accepted officially or tentatively as a wholesome food and feed by regulatory organizations in this and other countries. Most of the authentic strains studied in the United States apparently derive directly or indirectly from a parent stock sent to the University of Wisconsin by Bergius (Wisconsin culture No. 3) and also maintained at the Northern Regional Research Laboratory (NRRL Y900). The modified *Torulopsis utilis* cultures of Thaysen (NRRL Y1082 and NRRL Y1084) are perhaps next in importance.

Maintenance of *Torulopsis utilis* cultures is routinely carried out by accepted techniques of serial transfer and with little evidence of instability or variation. The lyophile process[46] has been found to be an excellent, labor-saving method of maintaining stock over long periods of time.

Two other organisms, *Candida arborea* and *Oidium lactis,* have been produced on a commercial scale in Germany and of these, at least the first has been extensively studied on a laboratory scale in the United States.

### Candida arborea

This organism seems also to have been referred to by the name *Monilia candida* and was grown at the I. G. Farbenindustrie plants at Wolfen and Bitterfeld in Germany. The utilization of pentoses and other desirable fermentation characteristics make it almost as favorable for industrial use as *T. utilis.* Its ability to maintain culture purity in open continuous-feed cultivation has been questioned, however.

### Oidium lactis

*Oidium lactis (Oospora lactis; Geotrichium candidum)* was used in the so-called Biosyn process at Wildshausen and at Lenzing and was also considered seriously for use by I. G. Farbenindustrie at other locations. Proponents based their claims for advantages of this organism on the ease with which it could be separated, washed, and dried with inexpensive equipment. It was hoped the threadlike mycelial form of growth would permit filtration and washing on ordinary screens. A disadvantage lay in the appreciable content of indigestible chitin in the cell substance. Its wide use and acceptance in the food and feed fields seems questionable.

A variety of other organisms has been proposed or reported to have advantages for use as a food or as an animal feed. The mycelium remaining from production of penicillin and other antibiotics has been used in animal feeds in this country. The residues and bacterial cell substance from the acetone-butanol fermentation also are known to have values in this respect quite aside from the high riboflavin content.

## DEVELOPMENT OF CULTURES TO THE PLANT STAGE

One of the remarkable features of torula-yeast production from spent sulfite liquor has been the ease with which large fermentation plants may be inoculated and maintained in continuous operation with apparent freedom from serious contamination problems. Few of the German torula-yeast factories are known to have been equipped with the elaborate pure-culture systems customary in the fermentation industry. Such systems apparently were used only for initial start-ups and after prolonged shutdowns, and preferred practice seems to have involved inoculation with yeast cream or yeast paste brought in from other plants or pure culture plants, such as that maintained at the Institut für Gärungsgewerbe.

Very few microorganisms grow as rapidly or are able to synthesize all growth requirements from simple compounds so completely as does *Torulopsis utilis. Candida arborea* duplicates the growth characteristics of *T. utilis* rather closely and is apparently the organism most commonly observed in association with it. Appreciable bacterial contamination is abnormal with properly controlled, continuously fed torula-yeast growth and, if found, is usually coupled with interruptions in operating routine or with lapse in proper sanitary-plant design and practices. Virus or phage infection which plagues some bacterial fermentations is apparently unknown in yeast plant operation.

## REQUIREMENTS FOR PRODUCTION OF TORULA-TYPE FOOD AND FEED YEAST

The use of suitable sources of low-cost carbohydrates is only one of numerous factors of prime importance in the efficient production of yeast. Very close control of growth conditions must be maintained continuously. These requirements are so exacting as to provide a fertile field for automatic control through modern instrumentation.

Basically the process consists of establishing active yeast growth in the fermentor, followed by feeding of carbohydrates and sources of nitrogen, phosphorus, and potassium, at increasing rates, until the maximum degree of yeast growth is maintained continuously at optimum rates for the particular substrate being used. Maximum efficiency in the yeast growth seems to be best achieved in the so-called "fine" or "emulsion" types of aeration and accompanied by considerable agitation. The exact quantities and qualities of aeration and agitation vary with the particular substrate. Once the continuous phase is established the yeast-containing effluent or "beer" is withdrawn at rates and volumes equaling that of the substrate being fed. Under ideal conditions, commercial fermentors have been operated for months without need for reinoculation or shutdown.

### The Concentration of Sugars and Their Rate of Feed

From the start of yeast growth, a primary objective is to feed sugars and other compounds utilized for yeast growth at such rates as to sustain maximum rates of reproduction by the yeast, at the same time maintaining minimum concentrations of the usable

carbon compounds in the fermentor. In effect, it seems desirable almost to starve the growing yeast but never to allow actual shortages to occur over and above the amounts that can be utilized.

The methods of controlling the sugar feeding may vary with the yeast plant. The German Waldhof plants use a quick method of analysis of total reducing substances,[42] while Thaysen[43] and Read,[29] in their pilot and semicommercial operations used a rapid method of determining the yeast in the fermentor and calculated from it the amount of sugar such amounts of yeast should be capable of using.

## The Air Supply

The amount of air supplied to the fermentor and the manner of its distribution are well known to be critical factors in yeast production efficiency by any process. This is especially true when working with foamy wood sugar solutions, such as spent sulfite liquor. De Becze and Liebmann[7] have reviewed this subject with special reference to molasses substrates. Porous stone diffusers, which deliver air in very small bubbles, have been quite successful and the pilot plants developed by Thaysen in England and by Rand in South Africa were both so equipped. The value of mechanical, "fine" aeration systems in molasses plants has not been so well reviewed in the literature. However, the wood sugar solutions have undesirable foaming properties which seem to prevent adequate aeration by systems other than those based on the principle of emulsion aeration by mechanical methods and which also incorporate mechanical control of foam. Chemical defoaming agents have, in most cases, proved unsuitable on these substrates.

By formation of emulsions having as much as two volumes of air for each volume of liquid, these mechanical systems give very high efficiencies of gas transfer at the air-liquid-yeast interface.

The amount of air required for efficient yeast growth is known to be critical. Too much air can be accompanied by increased carbon dioxide respiration, increased heat formation, and lowered yeast yields. Too little air allows anaerobic fermentation conditions to arise with loss of yeast yields in favor of alcohol production. Unfortunately, the efficiency of aeration is so varied in different substrates and with differing types and sizes of equipment as to make generalized specifications of air requirements quite difficult. Schmidt[35] has reported satisfactory aeration efficiency with the use

of 8 to 10 cu m of air per kg dry yeast produced in spent sulfite liquor by the Waldhof aeration process. He compares this value with amounts running three to five times that in the best aeration systems used in Germany up to 1940. These figures seem to be confirmed by de Becze and Liebmann[7] who showed minimum values equivalent to 35 cu m of air per kg (dry basis) in the manufacture of compressed yeast from molasses.

## The Inorganic Nutrients

Yeast requires nitrogen, phosphorus, and potassium in relatively large amounts and calcium, magnesium, copper, iron, and a number of other elements in lesser quantities. Most substrates or the dilution waters can provide a good part of these elements. Both cane and beet molasses may be especially valuable for yeast growth because of their high potassium content. Appreciable amounts of phosphorus and usable nitrogen are also present. Spent sulfite liquor is almost totally lacking in nitrogen, phosphorus, and potassium and some waste liquors are also short of magnesium for efficient growth. Thus the amount of nutrients required for yeast production may be quite dependent on the particular substrate used.

Working with South African molasses, Read[29] calculated a basic requirement, equivalent to 5.64 lb of ammonia and 1.76 lb of phosphorus, for each 100 lb of 95% dry yeast.

German design data for the Waldhof Standard plant, when recalculated on the basis of 40% yield on the reducing substances, called for 8.7 lb of ammonia, 1.29 lb of phosphorus, and 0.37 lb of magnesium per 100 lb of dry yeast. Earlier experience with the Waldhof process in 1940-1942 had shown need for about twice as much phosphorus.

Special yeast products, such as high-fat yeasts use different nutrient quantities than are indicated here for a normal high-protein yeast. The reader should consult the extensive patent literature for more detail on nutrient requirements for such products.

The exact form in which the inorganic elements are supplied is dependent on cost and availability and on their effect on growth.

## Temperature Control

Aerobic growth of yeast is accompanied by the liberation of large amounts of heat, so that it is very important to locate yeast

factories where suitable supplies of cooling water are available. Schmidt[35] showed appreciable differences in heat liberation by different substrates and attributed the effect in one case to a different fermentation mechanism in the utilization of pentoses accompanied by formation of nonutilized intermediates and the loss of more energy in the form of heat (see Table 32).

TABLE 32. *Heat Liberated by Yeast Growth on Various Substrates*

| Wort | Pentoses in wort approximate % | Heat evolved Cal per kg dry yeast |
|---|---|---|
| Wood hydrolyzate | 15 | 3,500 |
| Spruce spent sulfite liquor | 20 | 3,750 |
| Beechwood spent sulfite liquor | 90 | 4,600 |

The maintenance of an even temperature in the fermentor is recognized to be a prime factor in efficient yeast growth in the continuous phase. For normal growth of torula yeast, the temperature should be maintained closely in the range of 32° to 34°C, according to Schmidt. He found that increasing the temperature to 34° to 36°C reduced yeast yields by 5% and a temperature of 38°C produced 15% decreases. He noted that yeast required several days to recover from heat shocks above 38°C.

Thaysen[43] also emphasized these factors, but his development of the "thermophilic" strain of *T. utilis* permitted optimum temperatures in the range of 36° to 39°C and this factor is claimed to make plant operation in the tropics much more feasible.

## Control of pH

The control of pH can be quite important from a number of standpoints. Normally *T. utilis* plants operate in the pH range of 4.5 to 6.0, with the lower pH levels used where higher-temperature operation is required and where the sugars are fed in higher concentrations. Bacterial contamination can enter the picture at high pH and high temperature, and with the long holding times for given quantities of liquid substrate which usually accompany increases in sugar concentration above 2% in the medium.

The pH for operation may be controlled according to accepted practices. A commonly used method involves calculated addition of salts having acid or basic radicals not utilized by the yeast as a nutrient. As an example of this practice the degree of acidity may

be increased by feeding ammonium sulphate, a salt which leaves the sulfate ion in solution after the ammonium ion has been consumed as a nutrient.

## PROCESSES FOR PRODUCING PRIMARY-TYPE TORULA FOOD AND FEED YEASTS

The well-organized and detailed investigation of German industry immediately following World War II has given us unusual opportunities to gain knowledge which might otherwise have been disseminated slowly and piecemeal, if at all. A large gap prevailed between the basic knowledge covered in the extensive publications of Fink, Lechner, and Just and the industrial practice of firms, such as Waldhof and I. G. Farbenindustrie. Many years of expensive pilot-plant investigation might have been required to duplicate the findings of the research teams.

There has also been a generous policy of publication by the English Colonial Office in its description of the work carried on by Thaysen and associates to develop the yeast plant for Jamaica. The same can be said for papers describing new molasses yeast plants in South Africa and the United States.

### The Waldhof Standard Process

The unique features of the original Waldhof process are based on the patents of Claus,[6] Neuman,[24] Schmidt,[36] and Gade and Schulze.[16] There have been modifications and improvements as commercial experience was gained and the "standard" design, the basic principles of which are described here, is said to have also included some of the best features of the I. G. Farbenindustrie experience. Apparently the cooperative design work originated from the desire of the German government to establish the best system for use in new plants built to relieve the protein food crisis near the end of the war.

The Waldhof plants, for the most part, used beechwood sulfite liquor as the substrate, but operated equally well on spruce wood spent liquor and on the pentose-containing stillage after processing spruce-wood spent liquor by the alcoholic fermentation. Whey and some other substrates were considered satisfactory, but are not known to have been used extensively.

Spent sulfite liquor recovered from the pulp mill at pH 1.5 to 3.0 received brief aeration to remove free sulfur dioxide, then was

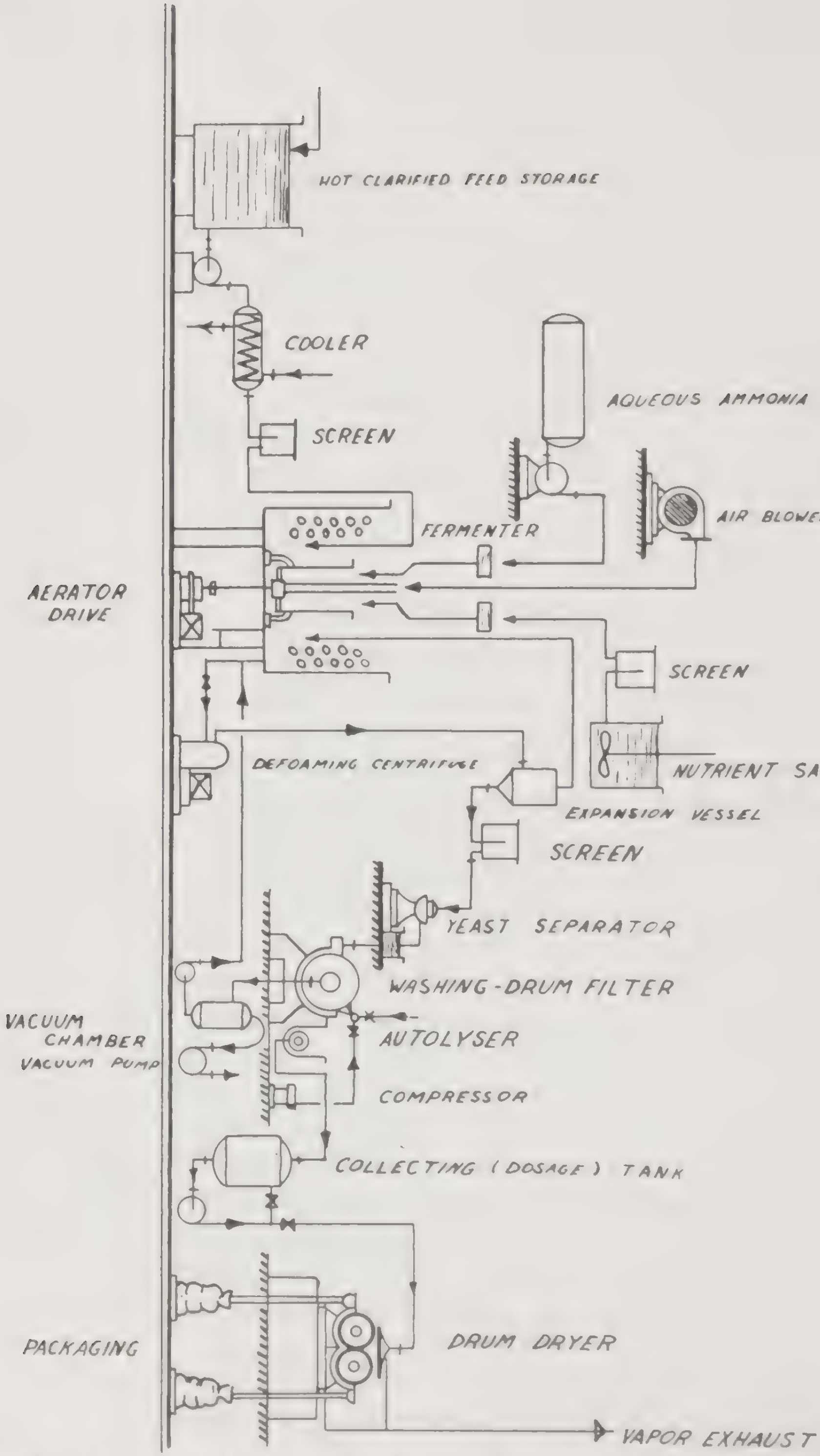



FIGURE 58. *Flowsheet of the Operation of the Waldhof Standard Plant*

neutralized with lime, using aeration for agitating and mixing, and finally sent to hot-liquor storage after clarification for 8 hours or more. The liquor thus pretreated had a pH of 5.0 to 5.5.

The flowsheet in Figure 53 outlines the individual operations in the yeast process from this point on. The steps covered in the flowsheet include continuous feeding of the waste liquor, ammonia, and nutrient solution to the fermentor where they are aerated in the emulsified state for optimum yeast growth. Simultaneously, the foamy, yeast-containing beer is withdrawn from the bottom of the fermentor, defoamed in a special centrifuge, screened, and the yeast separated centrifugally as a thin cream. The cream then passes to a vacuum drum filter for a combined washing and dehydration step. The paste from the filter is heated (autolyzed) and pumped to a double-drum drier, from which the yeast is delivered as 93 to 95% dry yeast flakes or powder.

#### Feeding the Fermentor

The clear, hot liquor is pumped through a heat exchanger to cool to the desired temperature, screened to remove traces of pulp fibers, and then fed to the yeast-growing vat (fermentor) at carefully controlled rates of flow.

Close control also governs the calculated flows of aqueous ammonia and supplementary nutrient salts solution fed to the fermentor simultaneously with the sugar-containing spent liquor. The chief function of the nutrient solution is to supply necessary amounts of phosphorus and potassium. However, these elements are usually supplied in the form of salts such as to provide more or less adjustment of the pH in the fermentor. Schmidt[35] considers about 5.5 the optimum operating pH for beechwood liquor in the fermentor.

#### The Fermentor

The fermentor (synonyms: yeast-growth vat, propagator, reactor) unit is the chief but not the only novel feature of the Waldhof yeast process. Recognition of the advantages of emulsified or "fine" aeration came to earlier workers and notably the Austrian inventor Vogelbusch.[44] However, in practice, the early forms of emulsion aeration were characterized by nearly unmanageable foam formation. The contribution of Waldhof lay in the development of quite simple methods for mechanical aeration to produce emulsions and of combined methods for foam control.

The open tank, as described by Saeman, Locke, and Dicker-

man,[33] is equipped with a central cylinder, having the aeration unit in the form of a wheel at the bottom. The air tubes form the pumping vanes of the aeration wheel. Air passes into the emulsion through the open tips of the air tubes. Turning of the wheel at high peripheral speed causes the newly introduced air to be dispersed or beaten into the emulsion. As the volume of the emulsion rises in the fermentor, it overflows the top and flows down through the central cylinder from which it is removed by the pumping action also produced by the revolving aeration wheel. Thus a high rate of circulation is maintained within the fermentor and there is no opportunity for accumulation of unmanageable foam in the properly operated units. The power required for aeration and agitation is about 0.5 kilowatt hour per pound of dry yeast. Performance features and design details of a unit, duplicating the commercial models, have been studied and described by Saeman.[32]

The high degree of aeration efficiency and of agitation and mixing action within the Waldhof fermentor permitted very rapid utilization of sugar. Holding times as low as 2.5 hours where reported from some sugar substrates. The standard plant was based on feeding beechwood spent liquor at such rates as to introduce new liquor in volumes equal to that of the liquid capacity of the fermentor each 4½ hours. In this case, the volume of the air-liquid emulsion approximated 3.3 times the liquid volume and the total tank volume was designed accordingly. For beechwood liquor, the fermentor design called for a diameter of 25 ft, a height of 14.75 ft, and an effective volume of about 36,000 gal at a working depth of 10.8 ft. Low-pressure air for the aeration unit was provided by a blower at rates up to some 900 cu ft per minute.

Cooling of the fermentor (see Figures 54 and 55) was accomplished by use of internal stainless steel coils with a total surface area of 1600 sq ft and also by undercooling the feed liquor.

DEFOAMING

The emulsion drawn from the fermentor was considered to be most efficiently handled in yeast separators if first defoamed in a special horizontal centrifuge (Schaumschleuder). This was so operated as to provide fast liquor drainage to the periphery and perhaps also a degree of pressure solution of entrained gases. Yeast was not removed from the liquor in this step as practiced by Waldhof.

YEAST SEPARATION

Defoamed liquor was passed through a screen and delivered to a number of Westphalia centrifuges which concentrated the yeast to cream having about one-tenth the volume of the beer. Usually, the yeast was washed by dilution and reconcentration in subsequent

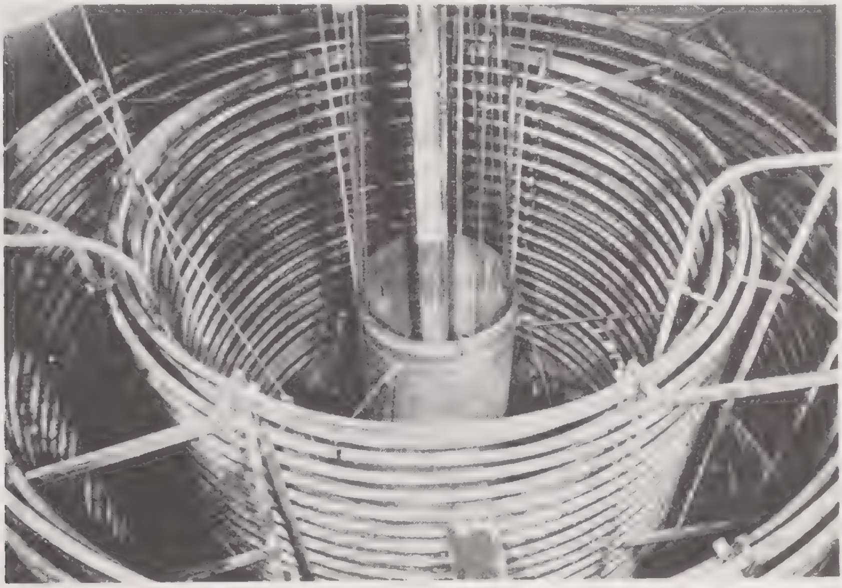


FIGURE 54. *Cooling Coils and Draft Tube in an Empty Waldhof Fermentor* (Courtesy—J. F. Saeman)

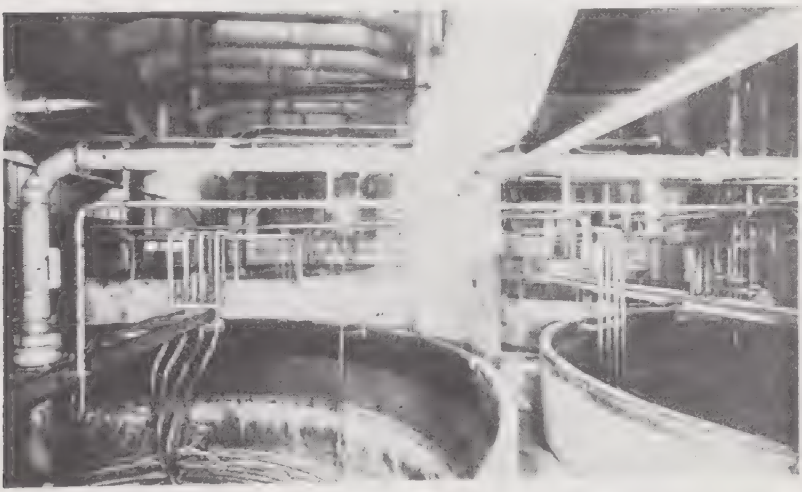


FIGURE 55. *Waldhof Fermentor Floor* (Courtesy—J. F. Saeman)

centrifuge operations, but the later standard plant design called for washing on the filter.

Further Dewatering of Yeast Cream

The yeast could then be dehydrated further to a paste running about 20% yeast solids by use of rotary vacuum filters so as to improve economy of final drying steps. Actually, much trouble was experienced with operation and cleaning of drum filters and other methods were being developed. Pavcek[26] refers to the I. G. Farbenindustrie "Schalschleuder" which combined the defoaming of Waldhof's "Schaumschleuder" with large capacity for yeast recovery in the form of slurry or cake with 20% solids. Another method of concentrating the yeast cream was seriously considered in Waldhof plants. This plan utilized special steam-heated evaporation units for the combined purpose of removing excess moisture from the cream and for short term heat autolysis of the yeast cells.

Drying the Yeast

The heavy cream from the centrifugal yeast separator or the heat autolyzed liquid yeast from the filter was dried either by drum or spray methods. The drum driers seemed to be favored in most plants.

## The S.P.M. Yeast Process at the Lake States Yeast Corporation

An American modification of the Waldhof yeast process has been described by Inskeep, Wiley, Holderby, and Hughes.[21] The S.P.M. process was developed by the Sulphite Pulp Manufacturers' Research League and was first placed in operation at the plant of the Lake States Yeast Corporation, Rhinelander, Wisconsin. A second plant to use the process is expected to be in operation by the end of 1953.

Certain engineering features shown in Figure 56 differ from those of the wartime Waldhof plants in Germany. Pretreatment of the sulfite liquor involves stripping of sulfur dioxide by means of countercurrent steam in a packed tower. The pretreated liquor is cooled in a spiral-type heat exchanger and passed to the fermentor. Cooling of the spent liquor-yeast-air emulsion is by external shell and tube exchanger rather than by internal cooling coils as in the Waldhof plants.

American-type yeast separators are used with simplified deaeration in a cone unit to reduce the air entrainment in the separator feed and without need for the high power consumption of the German "Schaumschleuder."

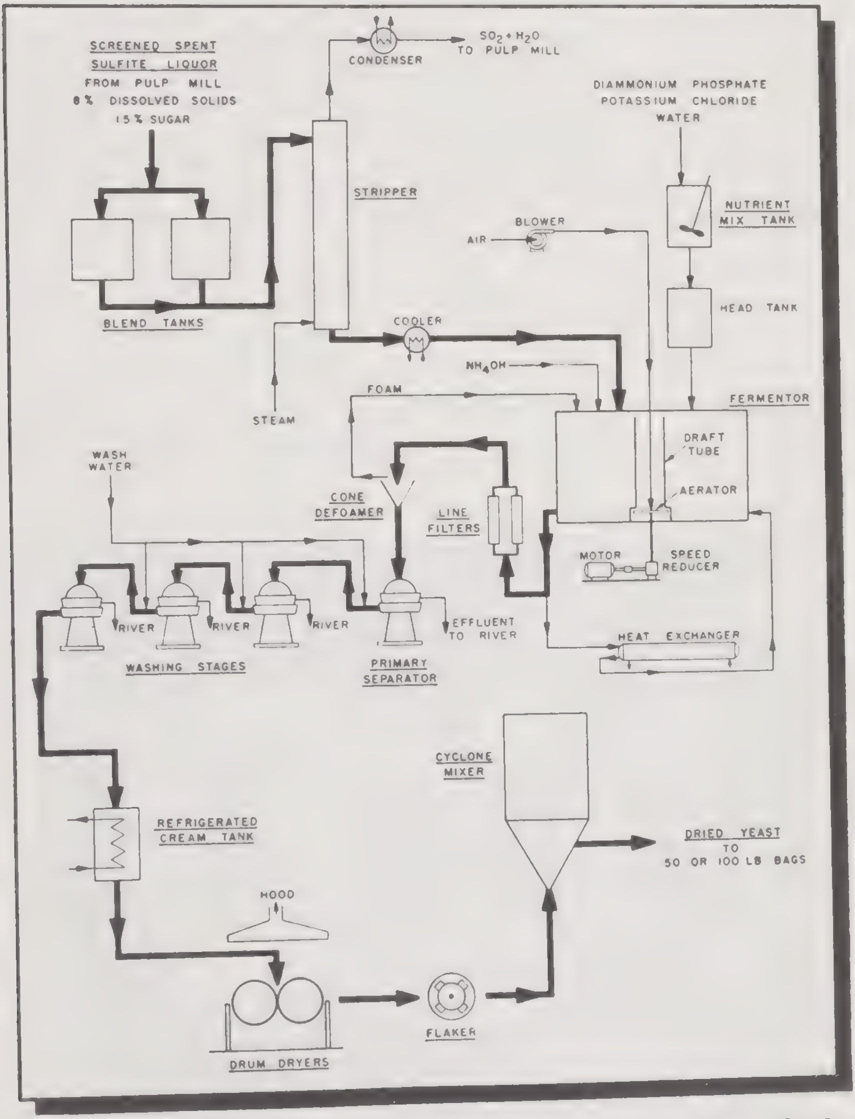



FIGURE 56. *Flow Diagram of S.P.M. Yeast Process at the Lake States Yeast Corporation Plant* [Reprinted by permission from a paper by Inskeep, Wiley, Holderby, and Hughes, *Ind. Eng. Chem.*, **43**, 1702 (1951)]

Extensive research has been devoted to the S.P.M. process for improving efficiency and reducing costs. Development of a number of the individual unit processes is continued while the plant is being operated.

## The I. G. Farbenindustrie Process

Actually, the various yeast plants operated by the I. G. Farbenindustrie used several different fermentation systems and the more recently built plants utilized the Waldhof principles. However, this company actively developed the automatic fermentation system of Scholler and Seidel[37] and used this design in plants at Dessau and in Ems, Switzerland. At least one other such plant is reported to be operating in Finland.

Novel features of the process involve a combined function of aeration and cooling by means of Seidel-type aerators, with a secondary pumping action operating on the principle of air lifts through a number of vertical, water-jacketed columns outside the fermentor. These aerator columns, in the form of large pipes, draw continuously from the bottom of the large fermentor tank and

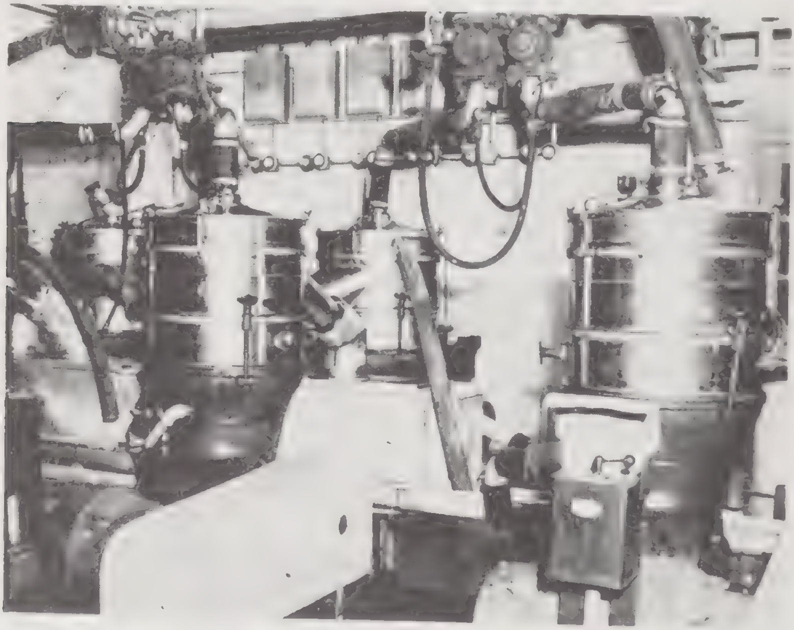


FIGURE 57. *Bank of Yeast Separators at the Lake States Yeast Corporation Plant* (Courtesy—Lake States Yeast Corporation, Rhinelander, Wisc.)

supply air under pressure in finely divided form through a stack of toothed rings in the base of each. The resulting air-lift effect raises and chills the foamy emulsion as it passes to the top of the water-cooled aeration tubes and back over the rim into the fermentor. The return flow at the top carries sufficient force to aid in knocking down any accumulation of foam. A central cylinder, open at both ends, promotes circulation within the fermentor much as in the Waldhof process. This system has been described by Saeman, Locke, and Dickerman[33] and further illustrated in reports by Pavcek[25,26] and by Bunker.[3,4] Details and economics of yeast production by this process are not so well known as for the Waldhof system and such reports as are available indicate need for improving some phases and unit processes.

### The Vogelbusch Aeration System

Early developments in the field of mechanical aeration systems were patented in a number of forms by Vogelbusch[44] and certain of these were adopted by the German yeast industry after 1936. As

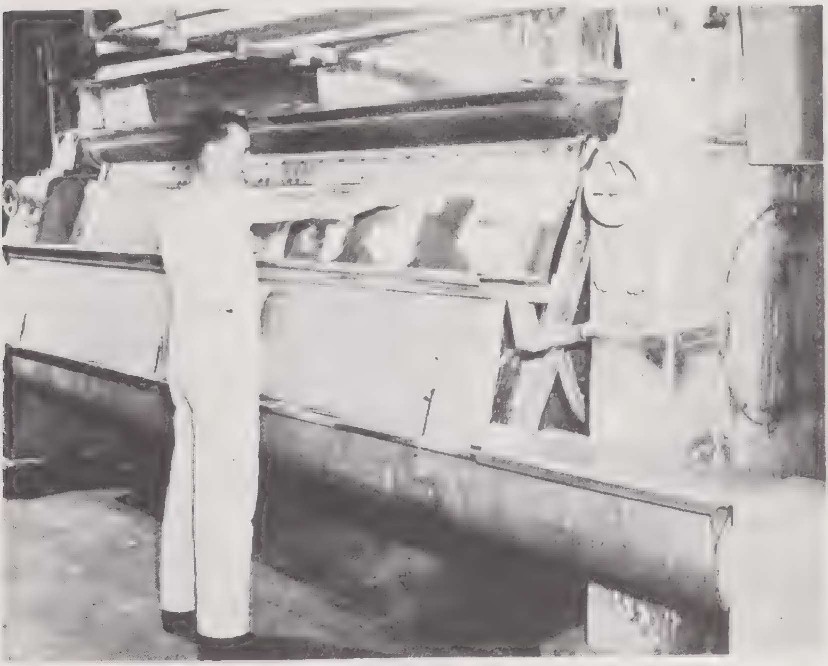


FIGURE 58. *Drying Yeast on Drum Driers* (Courtesy—Lake States Yeast Corporation, Rhinelander, Wisc.)

practiced for torula-yeast production in Aschaffenburg yeast plants, this system utilized a perforated hollow-blade propeller of wide diameter in the bottom of the fermentor. Air passing through the small holes in the surface of the moving blade sheared into extremely fine bubbles by contact with the liquid in the tank. Foaming trouble with sulfite waste liquor substrates apparently required use of supplementary mechanical or chemical defoamers at times and may have interfered with full yeast-production efficiency. This system is also described by Saeman, Locke, and Dickerman.[33]

## The British Colonial Food-Yeast Process

The wartime experimental program undertaken at the Teddington Research Station has been detailed by Thaysen[43] and by Bunker.[4] The pilot-scale studies in England have since been followed by construction and operation of a commercial plant in Jamaica and also have been the basis for design of semicommercial units in South Africa.[29]

Sugar used for yeast production by these methods is derived from cane molasses. The unit processes involved incorporate modifications and improvements to more or less standard bakers' yeast practice, such as to permit utilizing the special growth characteristics and advantages of *Torulopsis utilis* for continuous production of food yeast. Molasses is pretreated by dilution, acidified with sulfuric acid to pH 4.0, and heated to precipitate calcium sulfate and suspended organic matter. Stainless-steel fermentors have been found advantageous because of lessened solution of inhibitive quantities of iron. Aeration is accomplished by passing compressed air through porous ceramic stones in volumes sufficient to strongly agitate the substrate and, at the same time, meeting basic requirements for very finely divided bubbles. Foaming has been reported to be troublesome at times but manageable without resort to mechanical methods. Relatively high temperatures are used in the range of 36° to 39°C with the special strain of *Torulopsis utilis* var. *thermophilia.*

Production in Jamaica is understood to have averaged about 5 tons of dry yeast daily during the first year of operation and refinements are expected to raise this figure to about 12 tons a day.

Enlarged facilities of the Food Yeast Development Company, Ltd. at Merebank, South Africa, has permitted production to reach semicommercial scale with some 1,600 lb of yeast a day. This has

been achieved with use of a single small fermentor, 4 ft in diameter and 16 ft high.[23]

### The Carnation—Albers Process

Torula-yeast production by an interesting new method has been carried out in California since 1947 and culminates joint research and development by the Carnation and the Albers Milling Companies.[22] Primarily designed to produce vitamin and growth factor supplements for animal and poultry feeds, the yeast and the fermented molasses substrates are used without separation or preliminary drying in the fortification of feeds. By this innovation, it has been found possible to recover the soluble, extracellular vitamin products of the yeast as well as those retained within the yeast cells. Other novel features include modern industrial instrumentation and equipment.

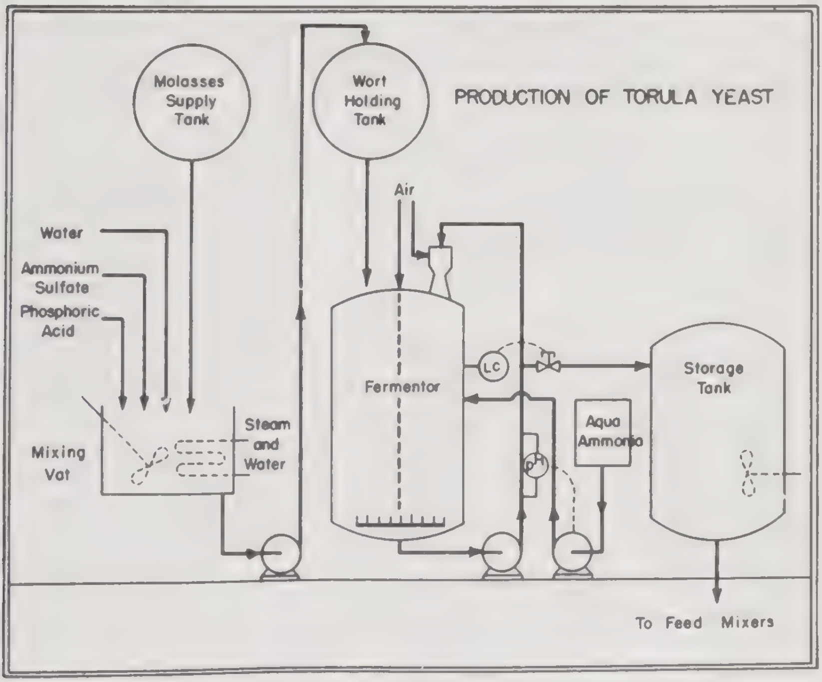



FIGURE 59. *Flow Diagram of the Carnation-Albers Yeast Process* [Reprinted by permission from a paper by MacKenzie, Noble, and Peppler, *Chemurgic Digest,* **8**, No. 9, 10 (1949)]

A flowsheet of the process is shown in Figure 59. Ammonium hydroxide nutrient feed to the fermentor is closely regulated by automatic pH control. A portion of the fermenting substrate is circulated through the pH-measuring chamber continuously and the controller actuates a pump, feeding aqua ammonia to hold the pH within 0.1 unit of the required value set at 5.0. The temperature and liquid levels are also controlled automatically.

Aeration is carried out by combination of a conventional perforated pipe grid in the base of the fermentor and of aspirators mounted on the closed fermentor head. The aspirators are water-jet eductors much like oversized laboratory filter pumps (see Figure 60). The yeast culture is pumped through the aspirator system at a rate of 500 gal per minute with simultaneous entrainment of some 100 cu ft of air per minute supplied under pressure to the pipe grid in the bottom of the fermentor.

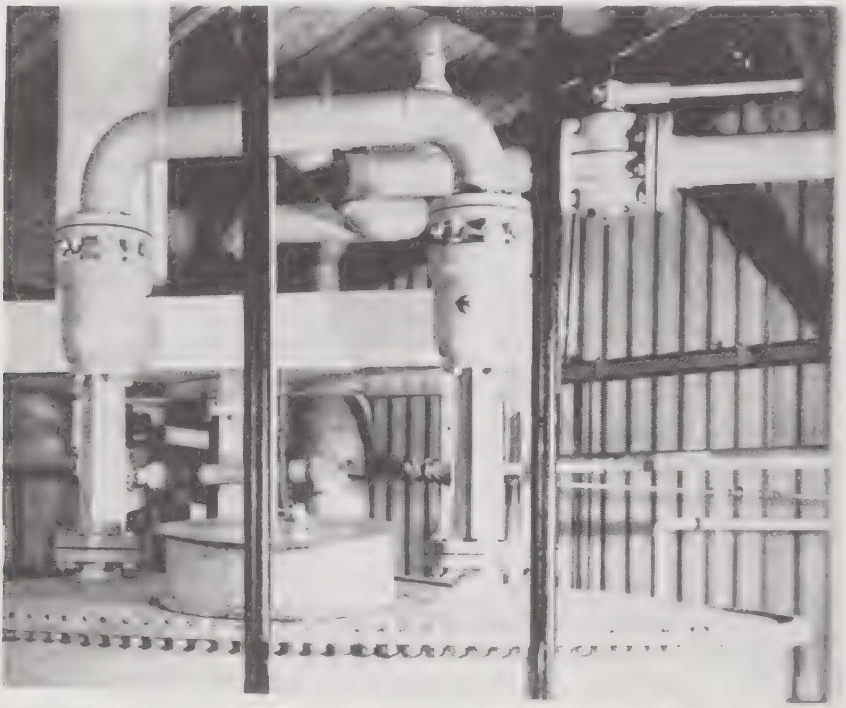


FIGURE 60. *Top of a Carnation-Albers Fermentor, Showing Aeration Jets* Reprinted by permission from a paper by MacKenzie, Noble, and Peppler, *Chemurgic Digest*, **8**, No. 9, 10 (1949)

## YIELDS AND RECOVERY OF YEAST

Data on yields of yeast obtained in commercial production, even where available, are somewhat difficult to evaluate and compare closely on different substrates, because of the diversity of analytical methods and special conditions under which they are reported. Practical methods for rapid analytical control of plant operations necessarily supplant slower research methods of analysis. Thus most reports on German production from spent sulfite liquor are based on average yields of 40% yeast (95% dry) in terms of the total copper-reducing substances. However, this waste liquor contains appreciable quantities of nonsugar reducing substances, such as calcium lignosulfonates, which serve to reduce the reported yield much below what it would be in terms of true reducing sugar. Further complication results from the fact that most of the practical fermentation substrates contain nonsugar carbon compounds, such as acetic acid, which can be readily utilized for yeast production. In such cases, the yield of yeast on the basis of true sugar is high.

Efficient yeast production on either molasses or spent sulfite liquor can be expected to utilize more than 90% of the true five- and six-carbon sugars present. Under such conditions, an over-all 45% yield of dry yeast on the sugar fed can be expected after including processing losses of 3 to 5% of the yeast inherent in centrifugal separation from the spent beer and in subsequent washing and drying operations.

## ANALYSES AND QUALITY OF THE YEAST

Food and feed yeasts derived from different sources are subject to some variations in individual qualities and characteristics due to the effect of the substrate and to species variations, but, when considered on an over-all basis, they are very similar in most respects. This is more apparent when comparing ranges of reported analytical data made on a large number of yeast samples, such as are shown in Table 33. Close control of growth conditions can be expected to give a fairly uniform product from any yeast plant and, within limitations, the yeast production can be controlled to yield certain desired characteristics. Most primary-type food and feed yeasts have compositions resembling that of brewers' dried yeast, with two exceptions: The bitter hop resins are not present and the thiamin content may be somewhat lower.

TABLE 33. ANALYSES OF TORULA YEASTS GROWN ON VARIOUS SUBSTRATES

(Moisture reported on as-received basis, all other analyses on moisture-free basis)

| | *Substrates Other than Spent Sulfite Liquor* | | | | | *Spent Sulfite Liquor Substrates* | | | |
|---|---|---|---|---|---|---|---|---|---|
| Chemical analyses, % | Citrus-peel juice (pilot) | Fir-wood sugar (pilot) | Pear waste (pilot) | Jamaican molasses (commercial) | Hawaiian molasses (commercial) | Wisconsin pulp mill A (commercial) | Wisconsin pulp mill B (pilot) | Wisconsin pulp mill C (pilot) | German pulp mill (commercial) |
| Moisture | 7.31 | 6.04 | 5.40 | 7.74 | 2.21[a] | 5.85 | 2.8[a] | 1.8[a] | 8.70[a] |
| Ash | 6.56 | 5.56 | 7.75 | 7.66 | 9.28 | 9.53 | 9.80 | 7.24 | 7.32 |
| Phosphorus, as P | 1.32 | 1.19 | 1.94 | 1.81 | 1.94 | 2.08 | 2.17 | 1.62 | 1.50 |
| Calcium, as Ca | 0.06 | 0.14 | 0.06 | 0.13 | 0.10 | 0.90 | 0.18 | 0.55 | 0.14 |
| Crude protein ($N \times 6.25$) | 47.1 | 54.2 | 47.9 | 46.7 | 51.2 | 50.4 | 55.6 | 55.0 | 50.2 |
| Crude fat, HCl prehydrolysis | 3.16 | 3.76 | 5.82 | 5.82 | 4.90 | 5.14 | 4.74 | 5.16 | 5.80 |
| Crude fiber, after prehydrolysis | 6.17 | 7.05 | 4.72 | 6.16 | — | 0.87 | 4.27 | — | — |
| Nitrogen-free extract | 37.0 | 28.4 | 33.8 | 33.6 | — | 34.0 | 25.32 | — | — |
| Vitamin assays, μg per g | | | | | | | | | |
| Thiamin hydrochloride | 13.7 | 6.9 | 14.5 | 9.9 | 9.6 | 5.6 | 13.4 | 7.7 | 3.4 |
| Riboflavin, microbiological | 48.2 | 80.8 | 44.9 | 39.8 | 78.2 | 47.9[b] | 53.5 | 56.8 | 50.4 |
| Riboflavin, fluorometric | 51.1 | 74.2 | 45.0 | 39.7 | 79.1 | 48.2[b] | 48.1 | 57.4 | 51.4 |
| Biotin | 6.4 | 2.3 | 3.1 | 3.4 | 3.4 | 2.4 | 2.57 | 1.2 | 1.86 |
| Niacin | 348 | 450 | 348 | 402 | 379 | 443 | 385 | 401 | 512 |
| Pantothenic acid | 110.7 | 134.4 | 40.1 | 50.8 | 30.7 | 41.7 | 50.9 | 90.8 | 18.0 |
| Pyridoxine hydrochloride | 37.8 | 38.3 | 36.6 | 43.3 | 42.1 | 35.5 | 50.9 | 38.8 | — |

[a] Laboratory-dried product.

[b] Average data from the same twenty-two samples.

Source: Reprinted by permission from a paper by A. J. Wiley, G. A. Dubey, B. F. Lueck, and L. P. Hughes, *Ind. Eng. Chem.*, **42**, 1830 (1950).

Food- and feed-yeast processes were originally developed for their protein and amino acid values in periods of food and feed shortage, but the 1940-1950 sales outlets in the United States were based more on the high concentration of the whole B complex of vitamins. Originally, the feed-grade yeasts were priced on the basis of the riboflavin content in excess of 40 $\mu$g per gram on the average. Other vitamins and yeast growth factors now are included in the feed sales picture, especially pantothenic acid, choline, niacin, and pyridoxine. Somogyi[41] has given an authoritative review of the whole range of nutritional values recognized in yeast up to the year 1944. Since then, there have been interesting developments in the field of new complexes and factors contained in yeast. Among these are streptogenin, glutathione, and one or more "unidentified" factors associated with the "whey" factor and the "animal protein factor" complex. Yeasts, in general, tend to be deficient or unbalanced in content of vitamin $B_{12}$. Supplementing yeast with this vitamin in recent feeding experiments has altered and improved the feeding worth in respect to methylating agents, such as methionine, choline, and betaine.

## RESIDUES AND WASTES

Disposal of fermentation-plant effluents is often a serious problem. For the two yeast substrates considered here in the first place, the production of yeast is in itself advanced as a promising solution, or partial solution, of serious disposal problems. Proponents hope to utilize the biological yeast-cell synthesis for conversion of surplus unused high-grade carbohydrates into useful and even profitable foods, feeds, and industrial raw materials. In the case of spent sulfite liquor, such utilization could result in marked relief to an increasingly acute stream-pollution problem whose large-scale economical solution was previously not possible. This is due to the sugars in this liquor being present in such low concentration as to be uneconomical for utilization by the older standard fermentive processes, yet too high for effective handling by known disposal systems.

Yeast production from sulfite waste liquor by efficient commercial practice removes 90 to 96% of the true reducing sugars and also similar proportions of the acetic acid present. In so doing, the pollution characteristics of the waste liquor, as measured in terms of the five-day biochemical oxygen demand (b.o.d.$_5$), are reduced

by more than 60%. The exact degree of b.o.d.$_5$ removal is dependent on the variable content of sugar breakdown products and other carbon compounds not utilized by yeast.

Molasses presents much the same picture. It has been an enormous waste-disposal problem in the past and still can be in times of surplus. The effluent from yeast growth processing has lost proportions of the original pollution characteristics, but still retains high b.o.d.$_5$ from presence of nonsugar carbon compounds not utilized after yeast growth.

While it may not always be a complete solution to pollution problems, yeast production provides a large step forward and promises to alleviate the problem to the point where standard disposal processes can handle the residual pollution properties.

## ECONOMICS OF PRODUCING PRIMARY-TYPE FOOD AND FEED YEASTS

The relative newness of these processes handicaps detailed and accurate evaluation of the economics of food- and feed-yeast production. War-time development in Germany and Switzerland was carried out under conditions difficult to evaluate even in Europe. Differences in costs for labor, nutrients, and other large factors are so great as to be of marginal value in interpolation to post World War II conditions in America. Information on production costs in Jamaica is not yet available and the plant for commercial production of yeast from spent sulfite liquor in Wisconsin, in the fourth year of operation, is still making process alterations to reduce costs. Preliminary engineering estimates indicate the costs of production can be expected to be somewhere in the range of 5 to 15¢ per pound from spent sulfite liquor and also from molasses when the price for molasses of 50% sugar content is less than 10¢ per gallon.

The value of large-scale sources of food and feed yeast to the market and the prices to be expected are also open to question. Under prevailing limitations on production capacity for secondary-type yeast, the market value for feed grades has held well above 8¢ per pound during and since World War II, with a ceiling around 15¢ per pound. Food grades of primary and processed secondary yeasts were priced to 50¢ per pound and more, while specialty products for pharmaceutical use were priced even higher. Considering the lack of authentic information, more detailed discussion

of the economics of food- and feed-yeast production is beyond the scope of this chapter. Schleef[34] has made an interesting study and evaluation of the economics of fodder-yeast production from spent sulfite liquor for conditions applicable to the Pacific Northwest and the reader is referred to this.

## PRESENT AND PROSPECTIVE USES FOR FOOD AND FEED YEASTS

Previous statements have indicated the volume of primary food- and feed-yeast production. The relative importance of the processes will necessarily be dependent on the size of the market which can be developed for these products.

German war-time production of yeast was based primarily on need for filling short supplies of protein foods and feeds. The yeast produced seemed to meet their requirements well. Postwar American protein markets are normally supplied by long-accepted meat and dairy proteins in the food field and by plant proteins in the animal- and poultry-feed fields. Competition by yeast will have to be based on quality factors which are yet to be recognized as superior.

English yeast production in Jamaica and that in South Africa were aimed, from the start, at eliminating human nutritional deficiencies in carbohydrate-rich and protein-starved areas of the tropics and the Far East. Production seems to be accepted and absorbed in increasing amounts by those channels to date. America apparently has only small need for yeast in this category for its own use, but new, large-scale American yeast supplies might find outlets in the export field.

American food and feed yeast enters the United States market as: (1) a rich supplementary source of vitamins, growth factors, amino acids, and mineral elements for animal, fish, and poultry feeds, and for substrates in other fermentation processes; (2) a source of vitamins and nutritional factors used in fortifying human food products; (3) a source of vitamins for pharmaceuticals; (4) a source of amino acids, protein fractions, and extracts for food and pharmaceutical use; (5) a source of enzyme materials for various end uses; (6) a source for yeast nucleic acids for pharmaceutical uses; (7) a raw material for various other fractionating processes in the pharmaceutical, chemical, and food industries.

American uses for food and feed yeast expanded rapidly during

World War II and almost reached the domestic capacity of secondary-yeast source supply. Primary-grown food and feed yeast has been produced in increasing quantities in the post-war period as new and growing outlets further increased the demand. Future large-scale expansion of demand seems probable, especially if the cost of yeast can be reduced materially from the levels existing during and before World War II.

## BIBLIOGRAPHY


1. Brahmer, H., *Svensk Papperstidn.,* **50**, 53 (1947).
2. Bunker, H. J., Private communications, November 25, 1948; March 8, 1949.
3. Bunker, H. J., U. S. Dept. Commerce, *Pub. Bd. Rept. No. 483* (1945).
4. Bunker, H. J., *Chemistry & Industry,* **1948**, 179; *J. Incorp. Brewers' Guild,* **1948**, 193.
5. Claassen, H., *Biochem, Z.,* **275**, 350 (1935).
6. Claus, W., German Patents 744,678 (1943); 752,725 (1944); 759,121 (1944).
7. de Becze, G., and A. J. Liebmann, *Ind. Eng. Chem.,* **36**, 882 (1944).
8. Delbruck, M., *Z. Spiritusind.,* **38**, 121, 137, 161, 235, (1915).
9. Delbruck, M., and F. Hayduck, *Die Gärungsführung in Brauerie, Brennerei und Presshefefabrik,* Berlin, Institut für Gärungsgewerbe, 1912.
10. Effront, J., *Ann. brass. dist.,* **25**, 166, 294, 362 (1927).
11. Feustel, I. C., and J. H. Thompson, *Western Canner and Packer,* **38**, No. 4, 60 (1946).
12. Fink, H., *Svensk Kemi. Tid.,* **50**, 177, 194 (1938).
13. Fink, H., and J. Krebs, *Biochem. Z.,* **301**, 137 (1939).
14. Fink, H., and R. Lechner, *Angew. Chem.,* **54**, 281 (1941).
15. Frey, C. N., *Ind. Eng. Chem.,* **22**, 1154 (1942).
16. Gade, M., and K. L. Schulze, German Patent 765,434 (1944).
17. Givartovski, R. V., *Edible Yeasts and Their Uses,* Izvestiya Tzentral Nauch. Issledovatel Inst. Pischevoi Prom., Separate (1930).
18. Givartovski, R. V., *Edible Yeasts,* Izvestiya Tzentral Nauch., Issledovatel Pischevoi Ukusovoi Prom., Separate (1930).
19. Harper, J. E., *Wood,* **2**, 20 (1947).
20. Heijkenskjöld, G. O. W., U. S. Patents 1,680,043 (1928); 1,703,272 (1929); 1,757,568 (1930); 1,881,557 (1932).
21. Inskeep, G. C., A. J. Wiley, J. M. Holderby, and L. P. Hughes, *Ind. Eng. Chem.,* **43**, 1702 (1951).

22. MacKenzie, E. B., W. M. Noble, and H. J. Peppler, *Chemurgic Digest,* **8**, No. 9, 10 (1949).
23. Masson, D. R., Private communication from F. O. Read, February 8, 1949.
24. Neuman, F., German Patents 752,723 (1944); 764,401 (1944); 729,842 (1942).
25. Pavcek, P. L., U. S. Dept. Commerce, *Pub. Bd. Rept. No. 4292* (1945).
26. Pavcek, P. L., *Wallerstein Labs. Commun.,* **13**, 369 (1950).
27. Prouty, C. C., *Washington State Agr. Exp. Sta. Bull. No. 484* (1947).
28. Rassow, B., *Z. angew. Chem.,* **32**, 313 (1919).
29. Read, F. O., *Proc. 21st Cong. South Africa Sugar Tech. Assoc.* (1947).
30. Rosenqvist, S. O., *Food Industries,* **16**, No. 6, 74 (1944).
31. Rosenqvist, S. O., *Svensk. Papperstidn.,* **45**, 506 (1942).
32. Saeman, J. F., *Ind. Eng. Chem., Anal. Ed.,* **19**, 913 (1947).
33. Saeman, J. F., E. G. Locke, and G. K. Dickerman, U. S. Dept. Commerce, *Pub. Bd. Rept. No. 7736* (1945).
34. Schleef, M. L., "The Economics of Fodder Yeast from Sulfite Waste Liquor," *State College of Washington, Bull. No. 7* (1948).
35. Schmidt, E., *Angew. Chem.,* **59**, 16 (1947).
36. Schmidt, E., German Patent 752,724 (1943).
37. Scholler, H., and M. Seidel, U. S. Patent 2,188,192 (1940).
38. Schwabe, K., *Zellstoff u. Papier,* **20**, 351 (1940).
39. Skoog, F., U. S. Dept. Commerce, *Pub. Bd. Rept. No. 2041* (1945).
40. Sperber, E., *Arkiv Kemi, Mineral. Geol.,* **21A**, No. 3, 1, (1945).
41. Somogyi, J. C., *Z. Vitaminforsch.,* Beiheft No. 4, 110 (1944).
42. Soxhlet, F., (Ross modification); see C. A. Browne and F. W. Zerban, *Sugar Analysis,* 3rd ed., p. 749, New York, John Wiley, 1941.
43. Thaysen, A. C., *Food,* **14**, 116 (1945); *Food Yeast,* (Pamphlet) Colonial Food Yeast, Ltd., 1944.
44. Vogelbusch, W., Austrian Patents 122,951 (1934); 123,393 (1934); 126,573 (1933); 127,365 (1934); 128,825 (1935); 136,969 (1934); 142,217 (1935); U. S. Patent 3,121,458 (1938).
45. Völtz, W., and A. Baudrexel, *Biochem. Z.,* **30**, 457 (1911).
46. Wickerham, L. J., and A. A. Andreason, *Wallerstein Labs. Commun.,* **5**, 165 (1942).
47. *Wood Pulp Statistics,* U. S. Pulp Producers Assoc., New York, July 1950.

# Part III. THE BUTANOL-ACETONE FERMENTATIONS

CHAPTER 11

# THE BUTANOL-ACETONE FERMENTATIONS

*W. N. McCutchan and R. J. Hickey*

The butanol-acetone fermentations are true fermentations, in the sense that they are anaerobic. The fermentations are brought about by various strains of *Clostridium acetobutylicum* and closely related species or variants.[6] The important neutral products produced by these organisms are *n*-butyl alcohol, acetone, and ethyl alcohol. Systematic names for these compounds are 1-butanol, propanone, and ethanol. *n*-Butyl alcohol is sometimes also designated *n*-butanol. In the subsequent discussion, it will simply be called butanol, with the understanding that 1-butanol or *n*-butyl alcohol is always meant.

The initial major developments of some of these processes for the conversion of carbohydrates to butanol, acetone, and ethanol on a commercial scale occurred at about the time of and following World War I. The industry would have developed eventually, but the war requirements for acetone provided a great impetus. Butanol was for a time of little commercial value and its storage and disposal constituted a major problem. Subsequently, however, it was found that butanol was a very useful chemical and some of its esters, such as the acetate, were used in large volumes in the manufacture of

lacquers for the automobile and other industries. Acetone became an item of secondary importance.

Much of the history has already been described in reviews, such as those of Gabriel,[18] Gabriel and Crawford,[19] Smyth and Obold,[67] Kelly,[27] Owen,[54] Prescott and Dunn,[58] and Porter.[57] A very recent review by Beesch[4a] discusses the current status of the processes. This chapter will stress particularly commercial operations and methods and will not dwell on details already adequately described.

The butanol-acetone fermentation of molasses will be presented in some detail since it has not been very extensively reviewed and has been a process of major importance. Its application volume has decreased recently because of the higher cost of molasses.

The major fermentation processes employed industrially, particularly in the United States, England, and Canada, have been the fermentations of corn and those of molasses. Among other raw materials, employed commercially, or of potential commercial application, or studied academically, are rice, potatoes, cassava, horse chestnuts, Jerusalem artichokes, processed corncobs, sulfite waste liquor, and wood sugar.[58]

Until about the time of World War II, the butanol produced commercially was largely a fermentation product. In recent years, a synthetic process,[84] has been developed and resulted in the production of a good grade of butanol from acetaldehyde quite cheaply. The synthetic method is based on the condensation of acetaldehyde to aldol which is converted to crotonaldehyde ($CH_3CH{=}CHCHO$) and then reduced to butanol. Acetylene, ethylene, or ethanol may be used to produce the acetaldehyde. The relatively cheap production of synthetic butanol is one of the reasons for the reduction in the volume of the butanol-acetone fermentations in the past few years. However, the fermentation processes continue to operate extensively.

Some acetone-butanol fermentations are made for the formation of riboflavin or of riboflavin feed supplements (Chapter 5, Volume II). Important raw materials for a fermentation in which riboflavin is of major importance are lacteal products, such as whey and skim milk. Corn or corn-rice media have also been employed where riboflavin was desired. These fermentations are quite critical as to iron content (Chapter 5, Volume II). In recent years, some organizations have generally found it more profitable to produce riboflavin by the *Ascomycete* processes employing

*Eremothecium ashbyii* or *Ashbya gossypii* rather than *Clostridium acetobutylicum*. This fact, along with the highly competitive synthetic butanol and more expensive molasses and grain, has resulted in curtailment of acetone-butanol fermentation operations in the United States and perhaps elsewhere.

International conflict has played a part in keeping grain prices high and avoiding sugar-cane surpluses so that cheap molasses, particularly high-test molasses, is not readily available as a raw material in some areas. Recent studies with sulfite waste liquor[68] have shown improved prospects for the use of sulfite waste liquor as an economical raw material.

## HISTORICAL

Butanol as a product of microbial action was probably first observed by Pasteur in 1861. He had been studying butyric acid-producing organisms and detected the presence of butanol. Among the early investigators in this field following Pasteur were Fitz, Trecul, Gruber, Grimbert, Beijerinck, Van Tieghem, Perdrix, Bredemann, Duclaux, and others. Prazmowski proposed the name of "clostridium" for these anaerobic spore formers, resulting in the genus *Clostridium* Prazmowski.[6]

The fact that acetone was also a fermentation product was not known until Schardinger made the discovery in 1905. The delay in this discovery is somewhat surprising since acetone is produced in quite appreciable amounts.

In 1909, there was considerable interest in Europe in the synthesis of rubber by the polymerization of butadiene or isoprene. In England, Strange and Graham, Ltd., obtained the services of Perkin, Weizmann, Fernbach, and Schoen to study the problems. After considerable investigation, it was decided that butadiene was a most desirable raw material for synthetic rubber and this diene could be produced from butanol. The fermentative production of butanol was then studied intensively. Some progress was made and Fernbach obtained an organism which produced acetone and butanol from potatoes. In 1912, Weizmann terminated his connection with Strange and Graham, Ltd., but independently continued work on the problem. In about 2 years, he found an organism which fermented a variety of starchy raw materials, including corn,[79] and produced butanol and acetone.

With the outbreak of World War I, acetone became a

critical commodity because of its use in the manufacture of the explosive, cordite. Strange and Graham, Ltd. contracted to produce acetone and attempted to do so by Fernbach's procedure and organism. The process was relatively unsuccessful. In 1916, Weizmann's organism and procedure were employed successfully, using corn as a raw material. Operations expanded and it was found desirable to operate the process in Canada, closer to the grain source, since shipping space was critical. This arrangement proved reasonably satisfactory. A plant was also started in India, but was not operated until after the war. Shortly after the United States entered the war, the Weizmann process was operated at Terre Haute, Indiana, for acetone production. Considerable quantities of acetone were produced, along with about twice as much butanol. The storage and disposal of the butanol presented a problem for some time. The signing of the armistice resulted in the closing of the fermentation plants for the production of acetone.

Intensive studies showed that the supposedly useless butanol could be used for the preparation of butyl acetate which was an excellent solvent for nitrocellulose lacquers. These lacquers found use as finishes in the growing automobile industry. The rapidity of drying and hardening of the lacquers was a boon to the automobile industry and soon butanol became a chemical in considerable demand, with acetone being somewhat of a by-product. Fermentation operations were again started and expanded as a result of the demand for butanol. Another plant was put into operation in 1923 at Peoria, Illinois. The Weizmann process was employed, using corn as raw material. Operations were enlarged at Peoria until in 1927 a total of ninety-six fermentors was in use, each fermentor having a capacity of 50,000 gal.[18] Later, with the use of molasses as a raw material for the butanol-acetone fermentation, other large plants were built in Philadelphia, Baltimore, Puerto Rico, and elsewhere.

When the butanol-acetone fermentation first became industrially important in the early twenties, attempts were made to use commercial blackstrap molasses as a raw material. At that time, many trials were made to ferment molasses with *Clostridium acetobutylicum,* using the Weizmann process. Mixtures of grain and molasses with various added nutrients, such as soybean meal and other proteins were studied. None was successful to any significant degree; results were generally relatively poor compared

with subsequent improved procedures for fermenting molasses.

In 1938, Müller[48] obtained a patent on a process by means of which he was able to ferment 4 to 6% monosaccharide solutions and produce solvents. To accomplish this, he employed a newly isolated organism which he designated *Clostridium propylbutylicum alpha*. It was isolated from decaying wood, but was later found in various woody fibers, including sugar cane. This organism yielded 25 to 30% of solvents, largely butanol, based on the total sugar. Müller made the important observation that a nutritional deficiency existed in previous molasses media. Müller's medium contained, in addition to the sugar, approximately 0.1% by weight of an ammonium salt, 0.1% of a phosphoric acid salt, and 0.2% of an insoluble alkaline metal carbonate, such as calcium carbonate. The carbonate acted as a buffer throughout the fermentation which was finished in about 48 hours. It was of interest that complex nitrogen was not required.

In order to utilize sucrose actively and rapidly by this method, it was found to be necessary to invert the sugar with acids or enzymes. This method marked the beginning of the successful butanol-acetone fermentation of molasses. Successful fermentation depended largely on the isolation and use of the proper organisms, the use of ammonia nitrogen, and the maintenance of an optimum pH value. However, it is doubtful if the specific organism and procedure described by Müller were ever used on a commercial scale. This was partly because sucrose-fermenting organisms were soon found.

In 1938 and later, Arroyo[1,2] and Owen[53] described butyl fermentation methods with inverted molasses, employing an organism first called *Bacillus tetryl* and later named *Cl. tetrylium* Owen.[6] This organism produced a high proportion of butanol and was employed industrially in Puerto Rico. Hildebrandt and Erb[23] patented a somewhat similar process in 1939, in which the organism used was identified as *Cl. celerifactor*. Ammonia was employed as a nitrogen source. Sucrose was not fermented well by either of these organisms.

In 1937, Woodruff, Stiles, and Legg,[86] and in 1938 McCoy,[40] reported processes which made use of a group of bacteria designated as strains of *Clostridium saccharo-acetobutylicum*. These were capable of fermenting molasses mashes, containing either sucrose or invert sugar, with yields of above 30% solvents, based on the sugar.

Inversion of the sucrose was unnecessary in contrast with the preceding methods. Nitrogenous nutrients had to be added in the form of degraded proteins, such as polypeptides, amino acids, urea, or ammonia or its salts. The amount of nutrients required varied with the raw material, but with cane-molasses media, about 0.7 to 1.7% of ammonia or its salt equivalent, based on the sugar, was required. It was necessary to maintain a pH of 5.0 to 6.2, and preferably of 5.5 to 5.85. This was done, when using a salt, by the initial addition of sufficient buffer, such as calcium carbonate, to neutralize any free acidity and furnish an excess to the extent of about 5 to 7% on the weight of the sugar. Calcium carbonate was cited as a preferred agent. Woodruff, *et al.*[86] described ammonia as a neutralizing agent, but warned against excessive additions. The optimum temperature was found to be 29 to 30°C. A small amount of phosphate was also usually necessary. The ratio of solvents produced was generally about 68 to 73% butanol, 26 to 32% acetone, and 1 to 3% ethanol. The described type of organism was used, to some extent, commercially. However, the actual rate of fermentation was slow, requiring about 72 hours. The organisms were also very susceptible to bacteriophage infection.

A patent was issued to Hall,[22] in 1939, for a process which employed an organism called *Bacillus butacone*. The spores were described as being unusually heat resistant, withstanding 100°C for 45 to 190 minutes. Both molasses and starchy media were fermented and complex nitrogenous nutrients were required. Patents were issued in 1938 to Arzberger[3] and to Carnarius and McCutchan[9] on the butanol-acetone process in which three new cultures were employed. These were identified as *Clostridium saccharo-butyl-acetonicum-liquefaciens* and the gamma and delta modifications or variants. These cultures gave consistent yields of about 30% total solvents based on the sugar, and fermentations were complete in 40 to 48 hours. The essential differences among these three cultures were based on their characteristic solvent ratios. The butanol formed varied according to the culture from 55 to 74% of the total solvents, while the acetone ranged from 22 to 40%. These cultures or subcultures have been employed by at least one large commercial producer for practically all of its butanol-acetone operations with molasses. The production procedures, involving molasses media, described in this chapter apply primarily to these or to similar strains of organisms.

## CULTURES

The organisms employed for the various butanol fermentation processes are *Clostridia* which are similar to *Cl. acetobutylicum* as described in Bergey's Manual.[6] The Weizmann type of organism is widely known as *Cl. acetobutylicum* Weizmann, and technically as *Cl. acetobutylicum* McCoy, Fred, Peterson and Hastings.[6,42] It has been widely employed in the fermentation of cereal-grain mashes. The sugar-fermenting organisms are similar, but they are often sufficiently different to result in patents on their use. Ideal cultures are evidently not readily found.[54] Industrial concerns have gone to special pains to patent operations with specific organisms which are carefully defined and differentiated in the patents. Many of these organisms are not available for general use.

In addition to variation in the morphology of the organisms, they may vary in carbohydrate substrates which they attack, rate of fermentation, type of nitrogenous nutrients required, and solvent ratios produced. Among the sugar- or molasses-fermenting organisms are, for example, *Cl. propyl butylicum* alpha of Müller,[48] *B. tetryl* described by Arroyo[1,2] and Owen,[53] and *Cl. celerifactor* of Hildebrandt and Erb.[23] These organisms necessitated the use of invert sugar for best operation since sucrose was not fermented readily. Other cultures have been found which ferment uninverted molasses. Among these are *Cl. saccharo-acetobutylicum*[36,40] and several variants of *Cl. saccharo-butyl-acetonicum-liquefaciens*[3,9] (see Table 35).

Studies on the isolation of cultures are still being made. A culture identified as *Cl. kaneboi* was isolated recently in Japan.[51] The isolation and the properties of the organism were described. The culture fermented both starch and cane-sugar media with good solvent yields.

## ISOLATION OF CULTURES

Molasses- and grain-fermenting cultures have both been isolated from many natural sources, such as soybeans, legume roots, gooseberries, wheat, and rye by modifications of the general procedure of Weizmann.[79] In general, they have been found associated with proliferating plant life, but not with decaying material. It has been noted that legume roots are especially good sources. This may be related to the storage of nitrogen in the soil by the nitrogen-fixing bacteria.

A suitable laboratory medium for development of molasses-fermenting cultures may be made from 10% barley malt in water by heating this to 68°C and holding for 30 min. This is then cooled and the coarse particles are strained out. The liquor may be supplemented with an ammonium salt and a buffer. It is sterilized for about 30 minutes, cooled, and inoculated with the root or other material. Pasteurization may be employed to reduce the content of vegetative organisms; the clostridia are relatively heat resistant.[22] Grain fermenting organisms are developed on potato or grain media.

The inoculated flasks of media may be incubated at 30 to 31°C for many of the sugar-fermenting clostridia and at 35 to 37°C for the starch-fermenting organisms. If butanol-producing organisms are present, gas evolution may occur after 24 to 48 hours and usually a distinct butanol odor will be evident after 2 to 4 days.

After 4 or 5 days, a small amount of the crude, sporulated culture is added to potato-glucose tubes. This medium consists of 25% wet potato mash, containing 0.2% calcium carbonate and 0.5% glucose. The tubes are "shocked" in boiling water up to 3 minutes and are then immediately cooled and incubated. Twenty-four hours later, a transfer is made to a new tube of potato medium, then two successive 24-hour transfers are made in sterile molasses medium for molasses cultures. The molasses medium contains 5.6% sugar as sucrose, with the following added nutrients, the percentages being based on the sucrose content: 5.4% ammonium sulfate, 5.4% calcium carbonate, and 0.2% phosphorus pentoxide in the form of a phosphate salt. For corn-fermenting organisms, tubes and flasks containing 5% corn mash are substituted for potato tubes and molasses flasks.

For the molasses process, the fourth generation flask is used to inoculate a quantitative molasses flask. This contains the same nutrients and buffer as the inoculum flasks and generally about 5.6% sugar as sucrose. These flasks are inoculated with about 4% by volume of a 24-hour inoculum. They are fermented for 72 hours at 31°C after which preliminary pH and Brix determinations are made. If these are favorable, solvents and generally acetone determinations are made. If these indicate that the impure culture contains desirable butanol-producing organisms, the cultures from both potato and molasses media are plated out on an agar suitable

for anaerobes. Several methods of obtaining anaerobic conditions can be used. A well-sealed desiccator containing wet, sprouting wheat in the bottom has proved successful.

The plates are incubated anaerobically at least 3 days at 31°C. Generally, they are then opened and incubated at least 2 days aerobically. The colonies continue to increase in size and take on characteristic color and form. The various types of colonies are then picked off the agar and placed in potato tubes and carried as before to the fifth generation flask for solvent analysis. These single-colony cultures may be pure. Different cultures may produce excellent to poor solvent yields and different solvent ratios under the same comparative conditions.

This general procedure can be modified in many ways. If an organism to ferment a higher sugar concentration is desired, the conditions of isolation can be varied to this end. If starch or pentose is to be fermented, development in starch or pentose media may be employed. This method might be referred to as a form of environmental selection.

## MAINTENANCE OF CULTURES

A number of cultures are mentioned in preceding and following sections of this chapter. Other cultures have been described in the patent literature.[4,5,39,41,49,50] These cultures are similar; they are anaerobic, motile, and have very similar cultural and morphological characteristics. All belong to the family *Bacillaceae,* and to the genus, *Clostridium.* The molasses-fermenting cultures generally require some ammonia nitrogen to proliferate and to produce full yields of solvents. They are also usually unable to utilize protein nitrogen alone and produce good solvent yields. Nor are they able generally to ferment starch with maximum yields even though the required ammonium salts are present. For example, Table 34 compares the results obtained from corn and from molasses, using the same molasses-fermenting culture and including an ammonium salt, a buffer, and a phosphate. The molasses medium contained 5.4% ammonium sulfate, 5.4% calcium carbonate, and 0.2% phosphorus pentoxide based on the sucrose. The corn medium contained 4.5% ammonium sulfate, 4.5% calcium carbonate, and 0.15% prosphorus pentoxide based on the dry corn meal. The culture used was a strain of *Cl. saccharo-butyl-acetonicum-liquefaciens.*[3]

TABLE 34. COMPARISON OF MOLASSES-FERMENTING CULTURE IN CORN AND IN NUTRIENT MOLASSES MEDIA

| Medium Type | *Original Medium* Sucrose % | Dry meal % | Final Brix | *Final Beer* Final pH | Solvents g per l | Yield based on dry meal % | Yield based on sugar % |
|---|---|---|---|---|---|---|---|
| Corn Meal | —— | 7.45 | 2.9 | 5.04 | 15.17 | 20.3[a] | —— |
| Molasses | 7.53 | —— | 2.2 | 5.69 | 22.65 | —— | 30.1 |

[a] Ordinarily, the corn-fermenting organism gives yields of about 26.5%, based on dry meal. No added nutrients are required when using the corn-fermenting culture.

All of the butanol-acetone cultures described show some differences. These may involve the ratio or type of solvents produced. Some organisms, for example, produce isopropyl alcohol instead of all or part of the acetone. This type of organism has often proved to be rather undependable and not very vigorous.

The organisms are all spore formers, and may be maintained on soil,[75] sand,[73] or on other inert materials. A satisfactory carrier is composed of 49% rich loam, 49% screened, washed sand, and 2% calcium carbonate. This mixture is sterilized thoroughly by intermittent heating. Generally, about 250 g are placed in a 500-ml Erlenmeyer flask for sterilization. A second- or third-generation culture in a molasses medium is selected for placing on soil. This is allowed to ferment 72 hours. Sterile soil in a flask is then saturated with the liquid culture. This is dried, finely crushed, and placed in a sterile flask. It is then tested for sterility from contaminating organisms by employing reasonably large samples as inocula for molasses media and for corn mash media. These are plated out aerobically the next 3 consecutive days. If no aerobic contamination is present, the soil culture is tested for solvent yield and ratio. Pilot runs may then be made. If these factors are satisfactory, one fermentor is set. If the fermentor results are good, more runs are made. If as many as twenty fermentors have given consistently good results, the soil culture is considered to be a good plant culture. Such cultures will keep on soil indefinitely, and are known to be viable after having been on soil for as long as 13 years.

## TEMPERATURE

The grain-fermentation processes, involving the use of the Weizmann type of *Cl. acetobutylicum* is generally operated at about 37°C, while the molasses processes are usually run at about 30 to 35°C. It has been pointed out by Carnarius[7] that cooling a molasses

fermentation to about 24.5°C about 16 hours after inoculation can result in a significant increase in the ratio of butanol to the other solvents. Temperature control generally involves cooling since heat is evolved during fermentation. Outside water curtains, i.e., water sprays on the exterior of the fermentors, have been commonly used for cooling. Some data on the effect of fermentation temperature on solvent yields are presented in Table 43.

## NUTRIENT REQUIREMENTS

There are different butanol-producing organisms which have been employed commercially for solvent manufacture. The nutritional requirements are certainly variable since some organisms will ferment corn mash while others will do so only poorly, although they may ferment a sugar medium, such as a nutrient-molasses medium quite well. Some organisms ferment both types of media,[51] and certain others, such as *Cl. saccharo-acetobutylicum,*[69] will ferment corn mash if ammonia nitrogen is added as a supplement. Ammonia nitrogen is preferred by some clostridia while others do best with complex-nitrogen sources (see Table 35). Organic-nitrogen sources as simple as asparagin have proved adequate for some butyl organisms.[81] Organisms, such as *B. tetryl,*[1,2] ferment invert sugar but not sucrose, while others[3,9,40,86] ferment both. The most useful grain-fermenting organisms are both amylolytic and proteolytic. The sugar fermenters, though their amylolytic and proteolytic powers may be poor, are still very useful.

Some of the nutritional studies have been reviewed by Porter[57] and by Prescott and Dunn.[58] Most of the reported investigations have been made with the Weizmann type of *Cl. acetobutylicum* and the applicability of these findings to the requirements of the various other butanol-acetone organisms is conjectural. A brief review of the nutritional requirements of *Clostridium* species, in general, was made by Porter[57] in 1946.

In 1940, Oxford, Lampen, and Peterson[55] employed a synthetic medium for studies on the nutritional requirements of *Cl. acetobutylicum.* Their studies, along with those of Rubbo and coworkers [62,63] and others,[57] indicated that biotin and *p*-aminobenzoic acid are essential factors for the growth of *Cl. acetobutylicum.*

Lampen, and Peterson[29] showed, in 1943, that biotin was essential for the growth of twenty strains of *Clostridium,* including nine strains of *Cl. acetobutylicum.* Of the strains of *Cl. aceto-*

TABLE 35. EXAMPLES OF SOME BUTANOL-ACETONE CULTURES AND THEIR APPLICATION

| Substrate type | Organism[a] | Nitrogen source | Optimum temperature °C | Solvents yield % on sugar | Solvents ratio B:A:E | Reference |
|---|---|---|---|---|---|---|
| Molasses | *Cl. saccharo-acetobutylicum* | $NH_3$ | 30 | 22.4 | 72.9:22.8:4.3 | 40 |
| Beet molasses | *Bacillus butacone*[b] | Complex N preferred | 37 | 36.5 | 70:28:2 | 22 |
| Invert molasses | *B. tetryl* | $NH_3$ | 35 | 30.0 | 70:20:5 | 2 |
| Invert molasses | *Cl. celerifactor* | $NH_3$ | 32–36 | — | 60:38:2 | 23 |
| Invert molasses | *Cl. saccharo-acetoperbutylicum*[b] | $NH_3$ | 30 | 29.6 | 75.6:22.4:2.0 | 4 |
| Blackstrap molasses | *Cl. saccharo-acetoperbutylicum*[b] | $NH_3$ | 30 | 31.2 | 69.5:24.9:5.6 | 4 |
| Blackstrap molasses | *Cl. madisonii* | $NH_3$ | 31 | 28.0 | 76.1:17.9:6.0 | 41 |
| Molasses | *Cl. saccharo-butyl-acetonicum-liquefaciens-delta* | $NH_3$ | 30 | 31.5 | 69.2:27.7:3.1 | 9 |
| Hydrol | *Cl. saccharo-acetobutylicum-α* | $NH_3$ | 30 | 33.0 | 67.5:28.1:4.4 | 34 |
| Corn hydrol | *Cl. acetobutylicum* | Complex N | 37 | 32.8 | 61.5:29.7:8.8 | 71 |
| Corn | *Cl. acetobutylicum* | Complex N | 37 | 31.8[c] | 61.5:29.8:8.7 | 71 |
| Corn | *Cl. saccharo-acetobutylicum* | $NH_3$ | 30 | 32.6 | 73.1:23.0:3.9 | 70 |

[a] Names employed in the references cited.

[b] Also ferments starch.

[c] In practice, using slop-back, solvent yields are generally about 34%.[43]

*butylicum,* seven also required *p*-aminobenzoic acid for maximum growth. They also found that *Cl. acetobutylicum* strain S9, supplemented with 0.002 $\mu$g per ml of biotin and 0.005 $\mu$g per ml of *p*-aminobenzoic acid, synthesized pyridoxin, pantothenic acid, folic acid, riboflavin, thiamin, and nicotinic acid. The riboflavin-producing ability of this type of organism is treated in Chapter 5, Volume II.

Reyes-Teodoro and Mickelson,[59] in 1944, studied the nutrition of several strains of saccharolytic butanol-acetone organisms and found that, as previously reported,[29] some required biotin and *p*-aminobenzoic acid in their nutrition, while others required only biotin. Thiamin, riboflavin, pyridoxin, nicotinamide, inositol, and pantothenic acid had no effect when added as supplements.

Commercial fermentations of importance have generally used complex substances, such as corn and molasses as raw materials. Corn mash constituted what appeared to be a complete medium for *Cl. acetobutylicum* Weizmann and commercial operation employed this medium. For satisfactory results with molasses media, special saccharolytic organisms were used,[3,9,48,86] since the Weizmann type of organism did not ferment molasses satisfactorily. Müller[48] pointed out that nutritional deficiencies were probably responsible for some of the poor results of early studies with molasses media. He employed ammonium and phosphate supplements with inverted molasses and showed considerably improved fermentative operation. His observations were subsequently adapted to fermentations employing sucrose-fermenting organisms.[86,3,9] Application of these general findings is described in other sections of this chapter.

## CONTAMINATION PROBLEMS

### Bacterial Contamination

Contamination of plant-scale grain and molasses fermentations has occurred from time to time. This problem has not generally been as troublesome in the molasses process as in the corn process. Samples from the initial laboratory inocula, up through the fermentors, are plated on aerobic agar plates and are checked for contaminants. The contaminating organisms have generally been rather limited in types. Lactobacilli have caused occasional troubles.[58] Quite often when contamination is found, the fermentation still proceeds reasonably satisfactorily in molasses media. With normal precautions, the problem is not great.

## Bacteriophage Infection

The bacteriophage infections of both corn and molasses butyl fermentations have been quite serious. Manufacturing plants have been shut down completely because of 'phage.

The first symptoms of the presence of 'phage, or virus, are extremely slow or sluggish fermentations in the corn process and cessation of gas evolution, along with slow fermentation, in the molasses process. In the molasses process, the medium appears dark colored, compared with the normal appearance, and microscopic examination shows either no organisms, or perhaps a few long, slender rods, with a "moth-eaten" appearance. After 24 to 48 hours, the fermentation may start again and produce solvents in moderate to poor yield.

The 'phage will pass through bacteria-retaining filters, such as the Berkefeld, and sintered glass filters. The bacteria-free filtrates will readily infect fresh cultures of the strain in which the 'phage appeared. 'Phages are fairly easy to isolate from natural sources, such as soils and river water. Some 'phages are quite specific, that is, they will attack one strain of organism and not another. Other 'phages may attack several strains.

It is generally possible to obtain 'phage-resistant cultures by growing them in the presence of increasing 'phage concentrations. A few 'phage-resistant organisms generally occur which are the basis of the resistance development. Examples of such methods were described by Legg,[32] Legg and Walton,[37] and McCoy.[41]

Legg's procedure,[32] based largely on the corn butyl process, involves repeatedly subculturing the organism in the presence of bacteria-free filtrates ('phage cultures) or "sluggish" butanol-acetone fermentation media. After each spore transfer, the bacterial culture is "heat-shocked" for 3 minutes at 100°C, just prior to inoculation with the 'phage. Incubation may then occur for 4 or more days or until sporulation begins. About ten such transfers are made and the resulting spore culture is usually immune to this particular 'phage.

The method described by Legg and Walton[37] applied to the sugar-fermenting organism alone. They found it unnecessary, and usually undesirable, to remove the vegetative forms of the bacteria before each successive subcultivation. The 'phage-susceptible culture was made to produce normal yields in a normal manner by effecting a series of subcultivations in the presence of the 'phage. The culture

was transferred during its most active stage and before sporulation occurred. Four to ten transfers were generally sufficient to produce a 'phage-resistant culture.

McCoy,[41] in a butanol-acetone process employing *Cl. madisonii*, uses a special medium composed of liver extract, tryptone, and glucose at pH 7.2 to produce 'phage-resistant strains. In this medium, the virus, or 'phage, at high dilution does not inhibit *Cl. madisonii,* while in a molasses medium, the culture is inhibited. About three to five transfers in this medium are used to obtain a 'phage-resistant culture. It is of interest that an increase in the number of liver-medium transfers is associated with an increase in the acetone proportion of the solvents produced.

It has not been uncommon in plant practice to find that a 'phage-resistant culture may succumb to another 'phage. The resistance development procedure is then again repeated and the resulting culture may then be resistant to two different 'phages. Most butanol-acetone plants maintain an assortment of 'phage-resistant cultures which may be employed to insure plant operations. Cleanliness of operation and of operational area is also quite conducive of 'phage-free operations.

'Phages have been found which attack many different bacteria and are not unique with the butanol-acetone industry. For example, actinophages are known which attack *Streptomycetes,* and the streptomycin industry has encountered some difficulty with such 'phages.[31]

## POSSIBLE YIELDS

In general, the maximum yields which have been reported for the molasses butanol-acetone fermentations have been about 29 to 33%, based on the weight of fermentable carbohydrate supplied. For example, 100 g of fermentable sugar will yield about 29 to 33 g of mixed solvents, consisting largely of butanol, acetone, and ethanol. The yields for corn fermentations are generally reported as based on dry meal. This usually averages about 26.5%, when employing *Cl. acetobutylicum* and "slop back" in the medium. Dry corn contains about 72% starch. Based on sucrose equivalence, the solvent yields from corn, under the conditions noted, are higher than the solvent yields from molasses, using a sugar-fermenting organism. The fermentation time averages 48 to 56 hours in an 8.5% corn-meal mash.

The classical solvents ratio produced by *Cl. acetobutylicum* McCoy *et al.* from corn mash is butanol:acetone:ethanol (B:A:E) = 6:3:1. Since acetone and ethanol are relatively unprofitable to produce by the butanol-acetone fermentations, while butanol is the primary item of economic importance, the manufacturer should produce the maximum yield of butanol at the expense of acetone and ethanol, if possible.

A great volume of patent literature has been concerned with the control of processes with new and different butanol-producing clostridia. These organisms are often characterized in great detail [22,34,40] in the patents in order to distinguish them from other organisms already described. Some of the more valued organisms are noted for the high proportion of butanol which they will produce. Butanol yields in the range of 70 to 76% of the total solvents have been reported for both the grain[69] and for the molasses[35,36,40,41,86] fermentations. An example[36] of the solvents ratio of such processes is B:A:E = 74:24:2. Table 35 shows some comparative data for a few of the patented processes.

The yield of solvents is limited by the concentration of butanol in the "beer." This limiting concentration is approximately 13.5 g per l. When the concentration has reached about this value, active fermentation will generally be retarded or stopped. It is, therefore, necessary to supply a low initial carbohydrate concentration in the medium, so that complete fermentation may be attained without allowing the toxic effect of the butanol to limit the solvent yield. The carbohydrate concentrations employed are usually in the range of 4.5 to 7 g per 100 ml.[2,36,72] *B. tetryl* did not appear to grow at 8.5 g per 100 ml of sugar.[2] Tarvin[72] fermented molasses media containing 7 g per 100 ml of sugar with supplementary nitrates which resulted in higher acetone and lower butanol yields. Over 40% of the solvents was acetone under some of the conditions. Weizmann, in a 1945 patent,[80] described the use of a mass-inoculation technique with *Cl. acetobutylicum* and was able to ferment media containing up to 10% sugar, leaving 1 to 2% of the original sugar unfermented.

A normal molasses fermentation will be completed in 40 to 45 hours. The use of stillage or "slop" in the medium results in a slightly faster fermentation. The yield of solvents is based on the weight of the total solvents obtained from a given weight of total sugar, usually calculated as sucrose. The yield is reported as per-

centage on sugar or as pounds per 100 lb of sugar. A 30% yield is considered reasonably normal from high-test molasses, compared with about 29% from blackstrap. Some minor handling and processing losses are generally incurred.

TABLE 36. TYPICAL SOLVENT YIELDS FROM HIGH-TEST MOLASSES, USING VARIOUS CULTURES

| Culture[a] | Molasses slop-back volume % | Initial sucrose g per 100 ml | Final Brix | Final pH | Total solvents g per l | Yield based on sugar % | Acetone in solvents % |
|---|---|---|---|---|---|---|---|
| E | None | 5.96 | 2.8 | 5.63 | 17.04 | 28.59 | |
| E | 40 | 5.90 | 3.4 | 5.73 | 18.42 | 31.22 | 21.7 |
| B | None | 5.88 | 2.8 | 5.71 | 17.70 | 30.10 | |
| B | 40 | 5.90 | 3.4 | 5.58 | 18.02 | 30.54 | 23.6 |
| D | None | 6.12 | 2.9 | 5.59 | 18.42 | 30.09 | |
| D | 40 | 6.14 | 3.7 | 5.49 | 18.70 | 30.45 | 27.4 |
| C | None | 6.89 | 3.4 | 5.58 | 19.96 | 28.96 | |
| C | 40 | 6.94 | 4.0 | 5.58 | 19.96 | 28.04 | 29.9 |
| A | None | 6.89 | 2.7 | 5.37 | 20.90 | 30.33 | |
| A | 40 | 6.94 | 3.8 | 5.44 | 20.90 | 30.11 | 39.0 |

[a] Culture A is a strain of *Cl. saccharo-butyl-acetonicum-liquefaciens.*[3] Culture C is a strain of *Cl. saccharo-butyl-acetonicum-liquefaciens-gamma.*[9] Cultures D, B, and E are strains of *Cl. saccharo-butyl-acetonicum-liquefaciens-delta.*[9]

Table 36 shows typical laboratory yields obtained from high-test molasses, using several cultures. Table 37 also shows the yields and solvent ratios produced by five of the molasses-fermenting cultures. The raw material was high-test molasses and, in each case, 40% molasses slop-back was used.

TABLE 37. SOLVENT RATIOS PRODUCED IN THE MOLASSES FERMENTATION

| Culture[a] | Sucrose g per 100 ml | Total solvents g per l | Solvent yield % on sugar | *Solvent Ratio* Acetone % | Butanol % | Ethanol % |
|---|---|---|---|---|---|---|
| B | 5.47 | 17.58 | 32.13 | 22.6 | 73.9 | 3.5 |
| F | 5.53 | 17.45 | 31.55 | 21.6 | 75.2 | 3.2 |
| G | 5.47 | 18.03 | 32.96 | 23.8 | 74.4 | 1.8 |
| C | 7.04 | 20.72 | 29.43 | 34.6 | 61.2 | 4.2 |
| A | 7.00 | 22.74 | 32.48 | 38.4 | 57.9 | 3.7 |

[a] Culture A is a strain of *Cl. saccharo-butyl-acetonicum-liquefaciens.*[3] Culture C is a strain of *Cl. saccharo-butyl-acetonicum-liquefaciens-gamma.*[9] Cultures B, F, and G are strains of *Cl. saccharo-butyl-acetonicum-liquefaciens-delta.*[9]

## ANALYSES

The general analyses of raw materials for industrial operation of the butanol-acetone fermentations involve moisture and starch determinations on grain and sugar content of molasses, which may include sucrose and invert-sugar determinations. Classical analytical methods, such as those published by the Association of Official Agricultural Chemists, may be employed.

Solvents are generally estimated as total mixed solvents along with the solvents ratio. The trend of the fermentation is checked by following the gas evolution rate, beer density (Brix), pH, and titratable acidity. Methods for estimating solvents and their ratios have been published.[11,21,25,66] Different organizations undoubtedly employ different analytical procedures and it is not within the scope of this review to go into analytical details. Davies and Stephenson[16] cited references for analyses of several compounds. It is possible to obtain quite accurate analyses of individual solvents from small beer samples by the use of the mass spectrograph.[52]

In brief, total solvents may be estimated, for example, by determination of specific gravity of distillates from the beer and comparing with a table of known values. The estimation of solvent ratio involves first the estimation of acetone by hypoiodite conversion to iodoform. By further distillation and salting out of the crude distillate, an anhydrous solvent mixture is obtained. The acetone content of the mixture is brought to a constant value of, for example, 25% by volume at 20°C by the addition of acetone or butanol as required. By a water titration to a turbidity endpoint, the ethanol content of the solvents is determined against known tables. The temperature must be critically controlled for this determination. Knowing the acetone and ethanol content, the butanol is determined by difference from the total solvents, since only these three solvents are present. The presence of isopropyl alcohol, of course, complicates the analyses.

Analytical studies which followed the course of the fermentation of grain by *Cl. acetobutylicum* in considerable detail were reported in 1932 by Peterson and Fred.[56] A series of excellent graphs were presented which characterized the fermentation in their hands. These graphs have been adequately reproduced in other reviews.[57,58]

## MECHANISM OF FERMENTATION

Although the mechanism of the alcoholic fermentation of sugar by yeast has been well established, along with the lactic acid fermentation by lactic acid bacteria and animal-tissue glycolysis, the intermediary metabolism of the butanol-acetone fermentation has not been completely elucidated. The status of our knowledge of the mechanism of the butanol-acetone fermentation was reviewed by Porter,[57] in 1946, and by Prescott and Dunn,[58] in 1949. Among the most recent studies on the mechanisms have been those of Cohen and Cohen-Bazire[12,13,14] in France and those of Rosenfeld and Simon[60,61] in Israel.

Attempts have been made to connect the three-carbon intermediates normally found in the alcoholic and lactic mechanisms with the butyl or butyric mechanisms. Simon[65] noted in 1943 that certain facts indicate that the butanol-acetone mechanism probably proceeds by other pathways than via the three-carbon intermediates. He pointed out that the customary three-carbon intermediates do not yield four-carbon products and that pentoses are not fermented by most yeasts, while, in contrast, the butyric-type organisms do ferment pentoses and produce the same four-carbon compounds as are obtained from hexoses and frequently in the same ratios.

Using washed cells of *Cl. acetobutylicum* Weizmann, Simon[65] found that modifications of functional groups at the $C_1$ position of glucose did not affect the four-carbon product appreciably. Where the CHO group of glucose was replaced by COOH, or $CH_2OH$, or where phosphate was introduced (Cori ester), butyric acid was found as a metabolic product. However, modifications at the $C_6$ position (e.g., galacturonic acid) usually resulted in acetic acid formation.

Simon[65] also found that phosphoglyceric acid, a mixture of phosphoglyceric acid and glycerophosphate, and D,L-glyceraldehyde were not attacked by the washed cells, and pyruvic acid yielded only acetic acid in the presence or absence of glucose. This appeared to be in contradiction with reports of Davies and Stephenson,[16] Cohen-Bazire and Cohen,[14] and others, who found butyric acid as well as acetic acid formation from pyruvic acid, but appears to be in agreement with the experience of Koepsell and Johnson[28] and Rosenfeld and Simon[60] The last investigators[60] found, however, that pyruvic acid yielded acetone and butyl products in good yield when *unwashed* cells were used. The only three-carbon compound which

yielded butyric acid[65] was glycerol. Consequently, in this case, a synthesis must have been involved. Simon[65] also observed that glycolaldehyde was not attacked, nor was sodium formate.

Using washed suspensions of cells of two *Cl. acetobutylicum* strains Davies[15] found that neither carbon dioxide nor hydrogen was evolved when the following potential intermediates were used: glyceraldehyde, phosphoglyceric acid, hexosediphosphate, lactic acid, formic acid, methylglyoxal, fumaric acid, butyric acid, $\alpha$-hydroxybutyric acid, $\beta$-hydroxybutyric acid, $\alpha,\beta$-dihydroxybutyric acid, glyoxylic acid, tetronic acid, crotonic acid, *trans*-$\gamma$-hydroxycrotonic acid, tetrolic acid, and vinylacetic acid. Acetoacetic and oxaloacetic acids were attacked with gas evolution. None of the compounds was reduced by hydrogen in the presence of active cell suspensions. Acetoacetic acid was decarboxylated to acetone. Butyric acid was reduced to butanol in the presence of glucose, but not of pyruvate.

In 1945, using heavy carbon ($C^{13}$) tracer, Wood, Brown, and Werkman[85] added labeled acetic acid ($CH_3C^{13}OOH$) to fermentations of corn mash by *Cl. acetobutylicum* and *Cl. butylicum.* Heavy carbon was detected in butanol, acetone, isopropyl alcohol, acetic acid, butyric acid, ethanol, and in carbon dioxide. The carbons of the butanol produced were about 50% $C^{13}$ and involved the 1 and 3 carbon atoms. Synthesis from acetic acid or a derivative was postulated. In both acetone and isopropyl alcohol, the central carbon atom was tagged. In another experiment, labeled butyric acid was employed with $C^{13}$ at the 1 and 3 positions. On addition of this acid to fermenting corn mash, heavy carbon was detected in ethanol, butanol, isopropyl alcohol, and acetic acid. The positions of the $C^{13}$ in the butanol was consistent with the presumed reduction of butyric acid. Similar evidence indicated that acetone was converted to isopropyl alcohol. Studies such as these indicate the presence of quite reversible systems.

Cohen and Cohen-Bazire[12,13,14] in 1949 and 1950 employed washed cell suspensions of a number of strains of *Cl. acetobutylicum* and related organisms for intermediary metabolic studies. All strains studied produced acetic acid from pyruvate, while butyrate formation varied from considerable amounts to none, depending on the organism employed. Arsenite inhibited butyrate formation, but not acetate formation. All strains produced lactate from pyruvate; the presence of fluoride resulted in decreased acetate and butyrate, while the lactate was increased. At the beginning of the pyruvate

conversion, only acetic acid was formed, while later butyrate formed, perhaps from acetoacetic acid which may be derived from acetic acid condensation. The same investigators[12] found in further washed cell experiments that lactate was not fermented alone, but in the presence of acetic acid, butyric acid was formed. Increasing the acetate concentration, while holding the lactate constant, resulted in increased butyrate. Butyrate formation was inhibited by fluoride, arsenite, and hydroxylamine. In later experiments, using washed bells of *Cl. acetobutylicum,* Cohen and Cohen-Bazire[13] found that $\beta$-hydroxybutyric acid was converted to acetone, butyric and acetic acids. Acetoacetic acid was also attacked by some strains of organisms, producing butyric and acetic acids, while others formed only acetic acid. Acetoacetic acid was reduced to butyric acid in the presence of lactate or pyruvate. In the presence of arsenite, increased butyrate resulted. Davies[15] found that acetoacetic acid was not reduced by cells actively fermenting glucose under slow decarboxylation conditions. The conversion of $\beta$-hydroxybutyric acid to other products might occur as follows:[13]

$$2CH_3{\cdot}CHOH{\cdot}CH_2COOH \longrightarrow 2CH_3{\cdot}CO{\cdot}CH_2COOH + 4H$$
$$CH_3{\cdot}CO{\cdot}CH_2COOH + H_2O \longrightarrow 2CH_3COOH$$
$$CH_3{\cdot}CO{\cdot}CH_2COOH + 4H \longrightarrow CH_3CH_2CH_2COOH + H_2O$$

The summation of these reactions would be:

$$2CH_3{\cdot}CHOH{\cdot}CH_2COOH \longrightarrow CH_3CH_2CH_2COOH + 2CH_3COOH$$

Studies with arsenite and fluoride showed in summary that arsenite prevented condensation of acetic acid to butyric acid. It also inhibited the reduction of $\beta$-hydroxybutyric acid and acetoacetic acid by lactate. Fluoride did not interfere with the condensation reaction, but the reduction reactions by lactate were inhibited, while reductions by pyruvate were not. Acetylphosphate was not detected.

Rosenfeld and Simon[60] showed in 1950 that pyruvate produced acetone in yields similar to those found in corn mash and in glucose fermentations. To obtain this result, they employed cell suspensions of *Cl. acetobutylicum* Weizmann in the presence of potassium and magnesium. The role of potassium had been studied previously by Davies.[15] Rosenfeld and Simon also showed that fluoride decreased hydrogen formation from pyruvate and increased the butyl products formation. It was concluded from their evidence that pyruvate was a precursor of both acetone and the butyl products.

Rosenfeld and Simon[61] then observed that since potassium and

magnesium promote acetone formation from pyruvate and since potassium and magnesium are involved in the conversion of pyruvate to phosphoenolpyruvate,[30] it was possible that this phosphorylated compound might be a reasonable intermediate in the butanol-acetone fermentation. It was found that phosphoenolpyruvate was fermented by *Cl. acetobutylicum* Weizmann cell suspensions to yield acetone and butanol or butyric acid. About 2.6 times as much butyl products were formed from phosphoenolpyruvate as from pyruvate, and about 50% more was found than was produced from pyruvate in the presence of fluoride. Fluoride promotes acetone formation and retards the fermentation rate.

The preceding information is a very brief representation of only a fraction of the studies which have been made in attempting to elucidate the mechanisms involved in the butanol-acetone fermentation. There are, of course, still many unknown factors to be worked out and hypotheses or theories to be proved. It is not within the scope of this book to go into detail in this interesting and somewhat controversial field.

## RAW MATERIALS

The primary carbohydrate raw materials for the butanol-acetone fermentation processes have been corn, including low-grade corn, blackstrap molasses, and high-test molasses. The characteristics of blackstrap and high-test molasses have been outlined in Chapter 3 and will also be discussed, to some extent, later in this chapter. Owen[54] has discussed uses of blackstrap molasses in several fermentation processes.

A large assortment of other raw materials has been employed in the plant or studied in laboratories. These include beet molasses, wheat, rice, horse chestnuts, Jerusalem artichokes, whey, oat hulls, corncobs, bagasse, flax shives, wood hydrolyzate, sulfite waste liquor, cassava, hydrol, assorted starches and sugars and even garbage.[24,35,36,38,39,57,58,66,68,71,74,83]

The corn employed in industrial operations is subjected to magnetic separation for removal of certain metallic foreign materials. It is then degerminated, the germ being used to produce corn oil. The degerminated meal is ground relatively finely and, when cooked in water for 1 to 3 hours, constitutes a complete medium for fermentation with the Weizmann type of *Cl. acetobutylicum*.

When blackstrap or high-test molasses is used for the fermentation, the media are generally deficient in available nitrogen and phosphate. Cultures, such as the *Cl. saccharo-butyl-acetonicum-liquefaciens*[3,9] type, can utilize ammonia nitrogen in their nutrition and do not need complex nitrogenous sources, such as are required by certain other strains (see Table 35).

Ammonia may be supplied as a salt, such as ammonium sulfate, or as aqueous ammonium hydroxide. If ammonium sulfate is used, a buffer, such as calcium carbonate, may be employed to neutralize the sulfuric acid remaining as the ammonium ion is metabolized. Ammonium hydroxide can be satisfactorily added at intervals to an active fermentation. Phosphate is supplied generally as "superphosphate," which is a calcium acid phosphate.

Stillage, or "slop," from preceding butyl fermentations may not be considered as a raw material in the same sense as molasses or grain in that it is not necessarily purchased, but is usually a fermentation by-product. It is a raw material from the angle of mash or medium composition. It may be employed to the extent of 30 to 40% of the total volume of the medium. The use of stillage in making up the mash is sometimes referred to as "slopping back."[53,71] Stillage is used for technical and economic reasons. The stillage or slop may add nutrients to media which are nutritionally deficient, such as high-test molasses. The slop addition lowers the requirements for supplementary nutrients. The solvent yields are also sometimes higher. The use of large volumes of stillage reduces plant requirements for water and also the total volume of stillage to be handled in disposal. This is a significant item, where the stillage is to be evaporated for use as a feed supplement.

In 1940, Stiles and Pruess[71] described what they referred to as a "criss-cross" slop-back procedure between ethanol and the butanol-acetone fermentations. Certain yield advantages were claimed.

The fundamental raw material for the production of butanol from molasses is sugar. From an economic standpoint, there have been primarily two important commercial sources of this sugar: blackstrap molasses, and what is known as high-test molasses. Blackstrap molasses is the mother liquor remaining after the refining of cane sugar. It averages about 52% total sugars, calculated as sucrose. It actually contains about 30% sucrose and about 22% invert sugar.

When there is an excess of sugar cane in the cane-growing

countries, the juice is expressed from the excess cane and concentrated to about 70 to 75% sugar in the presence of a little mineral acid. Thus the sugar is partially inverted to avoid crystallization on standing and contains about 50% invert sugar and 25% sucrose. The sucrose cannot be readily recovered as such. This product is known as high-test molasses.

High-test molasses containing 75% sugar is more desirable than the blackstrap containing 52% sugar because of economy in shipping costs and technical advantages. Nonfermentable solids, including salts are lower in high-test molasses. Blackstrap molasses contains many additional salts used to aid in the recovery of crystalline sucrose. In addition, the salts in the original cane juice are concentrated as much as the juice itself is concentrated. The types and chemical composition of these salts vary. This partially accounts for the wide variation in yields and nutrient requirements for different cargoes of blackstrap. The molasses-fermenting butyl organisms do not produce solvents from starchy grains to any great extent unless ammonia, or its salts, and a satisfactory buffer are present. With these supplements added to grain media, the yields are not as high as are obtained from molasses media containing equivalent carbohydrate.

Table 38 shows results obtained from various carbohydrate media, using molasses-fermenting culture A. Yields are compared with an unaided corn fermentation, using a Weizmann-type culture of *Cl. acetobutylicum.* The data of Table 38 probably indicate low proteolytic and saccharolytic powers of culture A.

The nutrient requirements for high-test molasses, based on the sugar content and using no slop-back, are well established at 1.4% ammonia and 0.2% phosphate calculated as phosphorus pentoxide. The ammonia is ordinarily added as ammonium hydroxide on a plant scale, but it may also be added as a salt, such as ammonium sulfate.

Table 39 illustrates the effect of the ammonium content on solvent yields from high-test molasses. In these examples, the ammonia was added as ammonium sulfate, using calcium carbonate for neutralization.

The effect of other ammonium salts on the fermentation is shown in Table 40. In each example, 35% by volume of molasses slop-back was used and an equivalent of 1.3% ammonia based on sugar Calcium carbonate buffer was included when an ammonium

Table 38. FERMENTATIONS OF VARIOUS MEDIA WITH A MOLASSES-FERMENTING CULTURE

| Culture[a] | Basal medium | Supplements added[b] | Solvents g per l | Dry meal g per 100 ml | Yield based on dry meal % | Sucrose equiv. g per 100 ml | Yield based on sucrose equiv. % |
|---|---|---|---|---|---|---|---|
| A | Corn meal | None | 0.07 | 6.85 | 0.10 | 5.2 | 0.13 |
| A | Corn meal | $(NH_4)_2SO_4$ $CaCO_3$ | 13.51 | 6.85 | 19.7 | 5.2 | 26.0 |
| A | Hydrolyzed[c] corn meal | $CH_3COONH_4$ $P_2O_5$ | 22.07 | 8.94 | 24.6 | 6.77 | 32.6 |
| A | Molasses | $CH_3COONH_4$ $P_2O_5$ | 22.55 | —— | —— | 7.03 | 32.1 |
| Corn fermenting culture | Corn meal | None | 22.12 | 8.43 | 26.2 | 6.38 | 34.7 |

[a] Culture A is a strain of *Cl. saccharo-butyl-acetonicum-liquefaciens.*[3]

[b] At concentrations of about 0.38 g per 100 ml of calcium carbonate and of ammonia, as ammonium salt, and 0.014 g per 100 ml of phosphate as $P_2O_5$.

[c] Hydrolyzed with HCl.

TABLE 39. EFFECT OF AMMONIA NITROGEN ON SOLVENTS PRODUCED IN HIGH-TEST MOLASSES MEDIA AT CRITICAL LEVEL[a]

| $(NH_4)_2SO_4$ employed based on sucrose, % | $NH_3$ equiv. based on sucrose, % | Sucrose g per 100 ml | Solvents g per l | Solvent yield[b] % |
|---|---|---|---|---|
| 3.9 | 1.00 | 5.91 | 16.82 | 28.46 |
| 4.3 | 1.10 | 5.87 | 16.48 | 28.07 |
| 4.7 | 1.21 | 5.89 | 17.80 | 30.22 |
| 5.0 | 1.30 | 5.87 | 17.92 | 30.52 |
| 5.4 | 1.40 | 5.95 | 18.42 | 30.95 |
| 5.8 | 1.50 | 5.97 | 18.42 | 30.85 |

[a] Where no ammonia nitrogen was employed, fermentation was extremely slow and solvent yields were negligible.

[b] Based on sugar as sucrose.

salt was used except for the acetate. The same culture was employed in all cases. In the table, the acetone analysis of the total solvents is shown. The butanol-ratio percentage is assumed to be the difference between 100 and the acetone-ratio percentage, except for 3 to 4% ethanol which is produced consistently and is considered normal.

TABLE 40. EFFECT OF VARIOUS AMMONIUM SALTS AT OPTIMUM CONCENTRATIONS ON ACETONE AND TOTAL-SOLVENT YIELDS

| Source of nitrogen | Solvents, g per l | Solvents yield, % | Acetone % of solvents |
|---|---|---|---|
| $(NH_4)_2SO_4$ | 17.00 | 29.82 | 22.2 |
| $NH_4Cl$ | 16.66 | 29.31 | 22.5 |
| $NH_4NO_3$ | 13.45 | 23.68 | 40.9[a] |
| $CH_3COONH_4$ | 17.34 | 30.42 | 27.5 |
| $NH_4OH$ | 18.59 | 31.58 | 22.4 |

[a] See also Tarvin[72] for effect of nitrates.

The ammonia and phosphate requirements for blackstrap molasses vary with the molasses. Usually less is needed than for high-test molasses. It is desirable to test the requirements in the laboratory for each cargo of blackstrap molasses. The ammonia requirements may be as low as 1.0% based on sucrose.

The use of slop-back is beneficial. "Slop-back" is a term used when some of the slop or residue from the stills is included in the molasses medium. Slop-back contains much protein and some

residual carbohydrates. The solvent yield is generally increased when slop-back is used to a limited degree. The slop contains by-products of fermentation, some of which are toxic. When slopping-back is practiced, the fermentations must be watched carefully. When slow fermentations begin to appear, slop-back should be discontinued for one complete cycle. The continued use of "slop-back" for the blackstrap-molasses process on a plant scale has been found to build up much residue detrimental to the fermentation.

Table 41 shows the effect of various volume percentages of "slop-back" in both blackstrap and high-test molasses media. Two different cultures were used. Culture A ordinarily yields 36% to 40% acetone[3] while culture B yields about 22% to 26% acetone.[9]

TABLE 41. EFFECT OF THE USE OF "SLOP-BACK" ON SOLVENT YIELDS FROM HIGH-TEST AND BLACKSTRAP MOLASSES

| Type of Molasses | Molasses Culture used[a] | Sucrose in slop-back volume % | original mash, % | *Final Beer* Brix | pH | Total solvents g per l | Yield based on sucrose % |
|---|---|---|---|---|---|---|---|
| Blackstrap | A | 10 | 7.50 | 4.8 | 6.40 | 20.59 | 27.4 |
| Blackstrap | A | 20 | 7.55 | 5.1 | 6.60 | 21.32 | 28.3 |
| Blackstrap | A | 40 | 7.63 | 5.2 | 6.45 | 22.19 | 29.1 |
| High-test | A | 10 | 7.38 | 2.0 | 5.56 | 21.84 | 29.6 |
| High-test | A | 20 | 7.38 | 1.9 | 5.64 | 22.36 | 30.2 |
| High-test | A | 40 | 7.53 | 2.2 | 5.69 | 22.65 | 30.1 |
| Blackstrap | B | 10 | 5.81 | 3.6 | 6.34 | 16.78 | 28.9 |
| Blackstrap | B | 20 | 5.81 | 3.8 | 6.43 | 17.84 | 30.7 |
| Blackstrap | B | 40 | 5.95 | 4.3 | 6.51 | 18.36 | 30.9 |
| High-test | B | 10 | 5.82 | 1.6 | 5.67 | 17.61 | 30.3 |
| High-test | B | 20 | 5.82 | 1.6 | 5.83 | 18.36 | 31.5 |
| High-test | B | 40 | 5.60 | 1.6 | 5.60 | 17.76 | 30.3 |

[a] Culture A is a strain of *Cl. saccharo-butyl-acetonicum-liquefaciens.*[3] Culture B is a strain of *Cl. saccharo-butyl-acetonicum-liquefaciens-delta.*[9]

Two other sugar sources of interest are beet molasses and hydrol. Hydrol is a residue from the manufacture of crystalline dextrose from corn starch. Beet molasses generally commands a higher price than does cane molasses, but gives excellent solvent yields and requires very little additional nutrients. Because of its higher price, it is not generally used for the commercial production of solvents. Examples of its use have been described by Legg and

Tarvin,[35,36] Loughlin,[39] and Hall.[22] Studies with sugar beet crowns have been made recently.[46]

Hydrol contains about 60% sugar. It has been used commercially to produce butanol when mixed with corn meal, using the Weizmann process and with cane molasses, using the sugar-fermenting organisms. In the second procedure, the same nutrients are used as with 100% cane molasses. Maximum yields are obtained if hydrol is substituted for molasses to the extent of about 10 to 20% of the total sugar, but even then the yields have often been lowered when compared with yields from cane molasses media. Methods of utilizing hydrol have been described by Loughlin,[39] Müller,[50] and others.[71,34,86]

Considerable laboratory work has been done on the fermentation of wood sugar obtained directly as wood hydrolyzate or as waste sulfite liquor.[38,66,83] There are certain interfering agents in waste sulfite liquor which can be removed by distillation and lime treatment. However, the concentration of sugar tolerated is comparatively low and no record is available showing what has been done on a commercial scale.

Attempts have been made for a number of years to ferment xylose produced by the hydrolysis of pentosans and cellulosic material, such as corncobs. Early results showed that at least 50% of the carbohydrate must be from such source as corn.[76] In 1948 Tsuchiya, Van Lanen, and Langlykke[74] described methods for fermenting a medium containing only xylose as its carbohydrate source. An assimilable nitrogen source, such as ammonium sulfate, was required, as well as phosphate, and finely divided iron to remove toxic substances, including copper. About 80 to 88% of the sugar was fermented. Corn steep liquor was employed as a supplement.

Whey and related lacteal products have also been subjected to the butanol-acetone fermentation process. Such fermentations were studied in Germany by Frey, Glück and Oehme[17] and by others in Japan and in the United States. A particular use of this process was developed in relation to riboflavin synthesis under special conditions (Chapter 5, Volume II). Even garbage can be fermented by the method of Jean[24] with a culture identified as *Cl. felsinae*. About 10 gal of solvents per ton of raw material was reported.

Other somewhat specialized modifications have also been described. Tarvin[72] used nitrates and other agents to modify solvent ratios. The use of buffers was described by Legg[33] and MacDonald.[44]

Kawano[26] claimed that the presence of acetates improved the fermentation and allowed the use of higher sugar concentrations. In 1945, Weizmann[80] employed rice bran in the molasses fermentation with *Cl. acetobutylicum,* and claimed that up to 10% sugar could be utilized. The residues were sources of vitamins.

In 1945, Gavronsky[20] described a procedure for the fermentation of nonsterile mashes. This involved the development of the culture in stages which utilized such relatively high inoculum percentages in later stages that the clostridial culture outgrew the contamination.

Recently, considerable work has been done by United States Government laboratories on the production of liquid fuels from farm waste materials. The investigators have discovered that the incorporation in the media of corn-steep liquor, ethanol stillage from grain fermentation, or a percentage of corn meal permitted satisfactory fermentation of hydrolyzate liquor from corncobs. Corn meal appeared to be the most effective.[64]

## FERMENTATION

### General Equipment

Molasses is generally stored in large outdoor tanks. It is moved by pumping and in very cold weather, provision for heat application to some pipe-lines may be necessary to reduce the viscosity and increase the flow rate. Other raw-material storage tanks must be available as well as mixing and slurry tanks for the preparation of media.

Corn is delivered in the shelled state and is stored in grain elevators. Grinding is done on roller mills and it is desirable to remove the germ for oil recovery. By the dry degermination system, 1 lb of oil can be recovered per bushel of corn. By the use of the wet degermination system, the oil yield is increased.

Inoculum-development tanks are required and will vary in size and volume ratio in different plants as noted later under "Seed Fermentation." The usual construction material is steel. Equipment must safely withstand sterilization under 15 psi steam pressure. Inoculum tanks should be equipped with a means of agitation, since the medium may be cooked and cooled in the tanks.

Cooking may be done batchwise in the smaller fermentation tanks or in agitated batch cookers. Continuous cooking is also employed. A continuous-cooking system for the grain-alcohol

process was first described by Unger.[77] A similar system applied to molasses mash was described in 1947 by Carnarius.[8] Quick sterilization is evidently essential in handling pentose media or toxic products may be obtained.[74]

Continuous-cooking systems make use of a continuously flowing stream of medium which is heated by steam through an injector at the beginning of the flow. The medium can either be made up in a slurry tank and fed to the steam injector or each ingredient can be fed in solution or suspension to the line at a point in the flow. The systems are so designed that they require enough time for the mash to reach the coolers at the desired temperature to sterilize the mash yet not overcook it. This is usually done by a series of tanks or by a pipe coil. For example, holding at 270 to 280°F for 2 to 6 minutes may be desired.[74] This cooker design is gaining favor because of its over-all efficiency, freedom from pockets, and ease of operation.

For the batch system, the conventional horizontal cooker, equipped with a 28 to 40 rpm agitator with rakes attached, may be used. This should operate safely at 30 psi steam pressure. A centrifugal mash pump is used to move the mash through the tubular heat exchangers and to the fermentors. Copper tubes may be used in the heat exchangers because of a low corrosion rate. Stainless steel may also be used. With copper, there is some danger of picking up enough copper to be harmful to the fermentation. This is less pronounced when "slop-back" is used. Presumably the copper is inactivated by a proteinaceous slop constituent. This is shown by the laboratory experimental data presented in Table 42. Similar data have been obtained with other copper salts. The interference of copper, along with other agents, has also been noted in the pentose fermentation.[74]

Fermentors may be of varied shapes and sizes; they are usually cylindrical, with hemispherical tops and bottoms, designed to withstand the sterilizing pressures. They should be pocket-free and easily cleaned and sterilized. All-welded construction is desirable for this reason. Since the inoculum and fermentor vessels are sterilized with steam, they must be maintained under sterile air or fermentor-gas pressure when cooling or vacuum may collapse the vessel. The relative amount of air or gas used is small and does not require large supply installations.

TABLE 42. EFFECT OF COPPER ON THE MOLASSES BUTANOL-ACETONE FERMENTATIONS

| Copper added ml 1% $CuSO_4 \cdot 5H_2O$ solution per l | Protein material added | 120 hours Brix | Final pH | Final solvents g per l |
|---|---|---|---|---|
| None | None | 2.3 | 5.71 | 16.84 |
| 1.33 | " | 7.1 | —— | trace |
| 1.0 | " | 7.3 | —— | trace |
| 0.67 | " | 7.3 | —— | trace |
| 0.33 | " | 3.1 | 5.36 | 14.67 |
| None | 35 vol % molasses slop | 3.0 | 5.76 | 16.55 |
| 1.33 | " | 3.1 | 5.54 | 15.17 |
| 1.0 | " | 4.1 | 5.34 | 11.86 |
| 0.67 | " | 3.2 | 5.37 | 14.44 |
| 0.33 | " | 3.1 | 5.26 | 14.77 |
| None | 8.3 g per l peptone | 3.0 | 5.48 | 15.58 |
| 1.33 | " | 8.0 | —— | trace |
| 1.0 | " | 3.7 | 5.71 | 13.39 |
| 0.67 | " | 3.7 | 5.58 | 13.90 |
| 0.33 | " | 3.0 | 5.51 | 15.28 |
| None | 9 g per l egg albumin | 2.3 | 5.70 | 16.66 |
| 1.33 | " | 3.0 | —— | 13.56 |
| 1.0 | " | 3.0 | —— | 13.95 |
| 0.67 | " | 3.0 | —— | 14.28 |
| 0.33 | " | 3.0 | —— | 15.38 |

Source: Data Courtesy of Commercial Solvents Corporation.

Continuous fermentation has also been studied,[82] but batch fermentations have generally been used commercially.

### Laboratory Development of the Culture to Plant Stage

Soil cultures having been established, their plant application must be considered. The molasses cultures may be started on potato medium. This medium is made by placing 250 g of potatoes in an autoclave and bringing the steam pressure up to 15 psi. Pressure is released and the potatoes are then removed and peeled. The mash is prepared by blending 5 g glucose, 2 g calcium carbonate, and the potatoes in a Waring blender with enough tap water to make 1 l of medium. About 15 ml of this medium is then dispensed per tube into 10 × ⅜ in. test tubes, which are plugged and sterilized for an hour at 20 psi steam pressure. For the corn-fermenting cultures, a 5% dry-basis corn mash is employed in the same type of tubes. Sterilization is for at least 2 hours at 20 psi.

To start either culture, a small amount of the soil culture, about 0.1 g, is placed in each tube of medium. The tubes are "shocked" by steaming in a cabinet steamer for 60 seconds. This aids in activating the spores. Immediately following the steam shock, the tubes are cooled in cold water for about 60 to 90 seconds or until the temperature of the contents is about 31°C for the molasses cultures and 37°C for the corn cultures. The molasses cultures are incubated at 31°C for 24 hours, after which the contents of each potato tube are transferred to a 500 ml Erlenmeyer flask, containing 300 ml of sterile molasses medium. This medium contains 5.6% of sugar as sucrose, calcium carbonate and ammonium sulfate each to the extent of 5.4% based on sucrose, and "superphosphate" to the extent of 0.2% as phosphorus pentoxide based on sucrose. Grain-fermenting cultures are handled similarly, except that grain mash is employed in place of molasses medium, and incubation is at about 36° to 37°C, instead of about 31°C.

The inoculated molasses flasks are incubated for 24 hours at 31°C. One of these is then used to inoculate 2,900 ml of the same medium in a 4-l Erlenmeyer flask. After another 24 hours at 31°C, this flask is used to inoculate the first plant inoculum vessel. To be satisfactory, this 4-l flask inoculum should show a highly active culture when a sample is examined on a hanging drop slide; the flask should exhibit rapid gas evolution and the pH should be about 5.4. It is essential that the fermentation should not be too far along or later development stages may suffer. This can be checked by comparing the Brix of the original mash with the Brix of the fermenting flask and with one that has been completely fermented. The Brix value for the inoculum should be about halfway between these extremes at the time of its use.

The corresponding corn flasks are incubated at 37°C and one is used to inoculate 2,900 ml of 6.5% corn mash in a 4-l Erlenmeyer flask. The same general controls apply to this flask as to the similar molasses-fermenting flask.

## Plant Fermentation

### Sterilization of Equipment

It is of primary importance to keep the operating equipment as clean and sterile as possible. The contaminating organisms thrive in mash which accumulates in pockets or crevices in the system; there-

fore, lines and vessels should be smooth and pocket-free. Welded lines and gate-type valves are used satisfactorily for the mash.

Steam is the generally employed sterilizing agent. The equipment should have ample provisions for the draining of condensate and venting of air, otherwise the temperature may be too low at points. There is no standard time nor temperature for sterilization. This must be learned by experience. In general, the system should be as simple as possible; steam should be applied to all portions of the equipment which are in contact with the mash. Sterilization control should be by temperature rather than by pressure. Chemical disinfectants may be used on equipment, floors, etc., to promote exterior sterility.

Seed Development

The laboratory culture may be available in 3-l quantities in 4-l flasks. The volume of the first plant generation may vary from about 75 to 800 gal. This is determined in part by the culture being used and in part by the preference of the technical staff of the manufacturer. A 3-l inoculum has been successfully used commercially to inoculate 700 gal of mash. This amounts to an inoculation ratio of about 0.1% by volume.

Molasses seed mash may be made up without ammonium salt, aqueous ammonia being added during the progress of the fermentation. However, it is more desirable, because of danger of over neutralization at this stage, to add to the cook an ammonium salt, preferably the sulfate, and a buffer, such as calcium carbonate. The amount of each should be about 5.4% based on sucrose. Additions will vary somewhat with the molasses used as noted in the section on raw materials. Usually about 0.2% phosphate as phosphorus pentoxide, based on sucrose, is also added in the form of a calcium phosphate.

A commonly used type of cooker is the batch cooker. The cooker is charged with the desired water, molasses, carbonate, ammonium salt, and phosphate for several seed tanks. This is then sterilized with direct steam under agitation. Care must be taken not to heat too long nor too hot. The maximum temperature should not be over 118°C, nor the time over 60 minutes. The mash is then pumped hot, through steam-sterilized lines, to the seed tanks where it is held at cooking temperature a short time and then

cooled with jacket water to 30° to 31°C. Cooking may also be done in seed tanks with agitators.

The vessel is now ready for inoculation. For control, the Brix and pH should be measured. The number of transfers of inoculum in the plant depends on the volume of the final fermentation vessel. These volumes are known to range from 50,000 to 500,000 gal. The percentage of inoculum used from step to step may vary somewhat with the culture and speed desired, but it usually runs from 2 to 4% by volume. Higher-percentage inoculum results in a shorter fermentation. Very high inoculum percentage is claimed by Gavronsky[20] to bring about such rapid fermentation that nonsterile mash may be employed in the final, largest-scale stages of development without risk of excessive contamination or loss.

The original Brix and pH on the seed tank are measured. The initial pH should be about 5.8 to 6.4. For an 800-gal seed, with a 3-l inoculum, pH, Brix, and gas-rate measurements are obtained about 24 hours after inoculation. The Brix should have dropped at least 1.5 points, the pH will be lower, possibly around 5.4 to 5.5 and using a Smith gas-rate tube, the gas evolved should replace at least 3 in. of liquid in the tube in 60 minutes. The culture should be examined microscopically by the hanging-drop method as already noted under laboratory development.

An aerobic agar plate should be made about 10 to 12 hours before the seed is used for inoculation. This should be incubated at 37°C and should show no aerobic growth. The seed, if normal, should be ready to use at an age of 26 to 28 hours.

The handling of the corn seed tanks is very similar. The mash is made up to 6.5% dry corn meal concentration and is cooked 90 min at 133°C. No added nutrients are required. The control analyses are the same as for molasses operations except that titratable acidity is run at intervals to check the progress of the fermentation, while pH and Brix are not run. Incubation is at 37°C.

### Fermentor Operation

Molasses is likely to contain a considerable amount of air. For this reason, it should be weighed. It is pumped from the storage tank to the scale tank and then to the mashing tun. This tun is previously partly filled with water, and with a metered amount of slop for slop-back. The molasses is weighed in and phosphate solution is metered in. Hot water from the coolers can be utilized

in the mash as a measure of heat economy. Because of the buffer salts present in blackstrap, it is possible to add a portion of the ammonium hydroxide required here before cooking. This cannot be done when using high-test molasses. The cook is made to volume and dropped to the cooker. Cooking for 1 hour at 107°C is generally adequate; excessive cooking is to be avoided. This also applies to the use of various continuous cooking methods which are mentioned under "general equipment." The hot cooked mash is then pumped through sterile lines and tubular coolers, i.e., heat exchangers, to the sterile fermentator; it arrives at a temperature of 30° to 31°C.

The fermentor is inoculated with about 2 to 4% by volume of active seed as noted previously. For control the original Brix and pH on the fermentor are obtained. The fermentor should start gas evolution at a fairly rapid rate within 6 to 8 hours after inoculation. The pH should drop and when it reaches 5.2 to 5.3, the addition of aqueous ammonium hydroxide can be started. This can be metered in increments with about 1 hour elapsing between additions. The pH should never be allowed to rise much above 8.5 or fermentation will stop.

At intervals, aerobic plates are made for checking contamination. Serious contamination may be evidenced by abnormally acid pH values and by cessation of gassing. It is economically important to recover as much unfermented sugar as possible from badly contaminated fermentors. Neutralization with sodium carbonate and reconstitution with some added molasses followed by recooking may be employed. If a considerable amount of solvents is present, the beer should be run through the beer stills with the stillage or slop being used with supplemental nutrients to charge another fermentor. A normal fermentor finishes its fermentation in about 40 to 45 hours and is ready to drop to the beer stills.

The mash and seed lines should be washed each day by pumping a cook of hot sterile water through the entire system when the daily run is finished.

When corn is used as the raw material, the meal is weighed, dropped to a mash tun partly filled with water at 65°C, the desired amount of "slop-back" is added, the material is mashed about 20 minutes and the mash is dropped to the cooker. It is usually cooked at 138°C for 90 minutes. Titratable acidity curves are run instead of Brix and pH to check the course of fermentation. Fermentation temperature is 37°C as for previous steps.

Table 43 shows the effect of temperature on the molasses fermentation, with three different strains of organisms. The effect of temperature on solvent ratio is quite important and was utilized in a patent issued to Carnarius[7] in 1938. In this process, the fermentation starts at 31°C; at 16 hours, it is cooled to 24.5°C. The butanol ratio is appreciably increased.

TABLE 43. EFFECT OF FERMENTATION TEMPERATURE ON SOLVENT YIELDS

| Culture[a] | Fermentation temperature °C | Final Brix | Final pH | Total solvents g per l | Yield based on sucrose % | Acetone in total solvents % |
|---|---|---|---|---|---|---|
| A | 30 to 31 | 3.6 | 5.53 | 21.30 | 31.00 | |
| C | " | 3.4 | 5.92 | 19.12 | 31.49 | |
| B | " | 2.8 | 5.80 | 16.64 | 31.04 | 22.8 |
| A | 33 | 3.2 | 5.51 | 21.80 | 31.73 | |
| C | " | 3.2 | 5.75 | 16.69 | 27.31 | |
| B | " | 3.1 | 5.78 | 16.47 | 30.61 | 26.1 |
| A | 37 | 4.8 | 5.53 | 16.12 | 23.46 | |
| C | " | 4.1 | 5.61 | 14.02 | 22.94 | |
| B | " | 3.3 | 5.34 | 13.92 | 25.87 | 38.3 |

[a] Culture A is a strain of *Cl. saccharo-butyl-acetonicum-liquefaciens*.[3] Culture C is a strain of *Cl. saccharo-butyl-acetonicum-liquefaciens-gamma*.[9] Culture B is a strain of *Cl. saccharo-butyl-acetonicum-liquefaciens-delta*.[9]

## RECOVERY OF PRODUCTS

The completely fermented medium is generally termed "beer." This beer may contain 1.7 to 2.4 g of total solvents per 100 ml, depending on the initial carbohydrate concentration and on the temperature and culture used. The ratio of ethanol, butanol, and acetone will also be variable. The beer may be temporarily stored in a large holding vessel, or "beer well," prior to distillation.

The beer is first run through a beer still which strips off the solvents. This is a continuous-type still of about thirty perforated plates in which the beer enters near the top and flows down through upflowing steam which vaporizes the solvents. These somewhat crude, mixed solvents are condensed by cooling and yield a concentrate of about 40% by weight of total mixed solvents. These solvents are then separated by fractional distillation. A high-boiling fraction is also obtained which is called "yellow oil" and is composed of higher alcohols and esters.[45]

Christensen,[10] in 1939, showed that the butanol-acetone and the ethanol fermentations could be operated separately with a combination of products on recovery to yield a power-alcohol product which blends readily with hydrocarbons.

Among the by-products of the butanol-acetone fermentation process are the fermentation gases. The amount produced from cultures giving the higher butanol ratios is about 4.7 cu ft per lb sucrose. About 67% by volume is carbon dioxide, the remainder being hydrogen. Cultures producing higher acetone percentage yield a greater proportion of hydrogen in the evolved gases. These gases have been used for synthesizing both methanol and ammonia. The carbon dioxide is often converted to dry ice (see Chapter 3). The hydrogen may be burned or used as an industrial chemical.

## BY-PRODUCTS AND WASTES

As in most fermentation processes, the disposal of stillage, or slop, has at times been a serious problem. It was discovered by Miner[47] in 1940 that a very large amount of riboflavin along with other B-vitamins was produced by the acetone-butanol fermentation of molasses. A process was worked out for concentrating the stillage by distillation to a syrup which was evaporated and dried. Many tons of this dry material have been and are being used as a vitamin supplement for feed stuffs (see Chapter 5 of Volume II).

The stillage is taken directly from the beer stills at a solids content of about 2.4 g per 100 ml. This content will vary, of course, with the type of molasses and with the concentration in the mash. The stillage is concentrated to about one-tenth the original volume in triple- or quadruple-effect vacuum evaporators. The con-

Table 44. RIBOFLAVIN IN RESIDUAL SOLIDS FROM MOLASSES FERMENTATION

| Curture[a] | Slop-back % | Total solvents based on sucrose, % | Acetone % | Riboflavin in final solids μg per g |
|---|---|---|---|---|
| A | None | 30.47 | 39.4 | 102 |
| A | 40 | 30.71 | 40.1 | 95 |
| B | None | 31.35 | 27.4 | 79 |
| B | 40 | 31.85 | 26.7 | 71 |

[a] Culture A is a strain of *Cl. saccharo-butyl-acetonicum-liquefaciens*.[3] Culture B is a strain of *Cl. saccharo-butyl-acetonicum-liquefaciens-delta*.[9]

centrated syrup is then dried by spray or drum drying. This constituted one of the early commercial riboflavin concentrates for feeding and was sold with 60 to 100 $\mu$g riboflavin per g. The product was sometimes fortified to form a more potent riboflavin concentrate.

Cultures have some effect on the riboflavin production. Cultures giving a higher acetone ratio have sometimes yielded more riboflavin. This is shown in Table 44.

Of course, the solids in the slop have a decided effect on the concentration in the final feed. Table 45 shows the effect of the use of blackstrap versus high-test molasses as a raw material.

TABLE 45. EFFECT OF TYPE OF MOLASSES ON RIBOFLAVIN POTENCY IN RESIDUE

| Blackstrap in total molasses Volume % | High-test in total molasses volume % | Yield of total solvents based on sugar % | Total solids g per 100 ml | Riboflavin in solids 2 $\mu$g per g |
|---|---|---|---|---|
| None | 100 | 30.95 | 3.47 | 60 |
| 10 | 90 | 32.16 | 3.59 | 64 |
| 50 | 50 | 30.36 | 4.24 | 58 |
| 70 | 30 | 31.17 | 4.49 | 53 |
| 100 | None | 30.40 | 4.96 | 49 |

The total solids shown in Table 45 are somewhat high, partly because ammonium sulfate and calcium carbonate were used instead of ammonia. However, the increased solids due to the use of blackstrap is typical.

The feed supplement produced by this evaporating and drying process contains 28 to 30% protein. Since nothing is employed in the medium except molasses, ammonium hydroxide and some inorganic salts, this protein is evidently largely of bacterial origin.

## ECONOMICS AND COMPETITIVE PROCESSES

A favorable economy for the production of butanol from molasses or grain is dependent mostly on the cost of the raw materials. The principal competitive process is the synthetic butanol process. The production of fermentation acetone cannot compete with acetone synthesis in price. This leaves butanol as the key product to consider. As a rule, 1 lb sucrose, in the form of molasses, is cheaper than 1 lb sucrose equivalent in the form of corn. Nor-

mally butanol can be produced cheaper from molasses than from corn.

Synthetic butanol from ethylene is quite a competitor for fermentation butanol. The synthetic process requires a high initial capital expenditure, but production costs do not fluctuate as widely because of more stable raw-material costs. The production costs for synthetic butanol or acetone are not available. It is significant that by far the greatest amount of acetone is made synthetically and the price is low. Considerable butanol is made synthetically, but the exact figures are not available. There is every reason to believe, however, that the manufacture of butanol by fermentation will continue to be a very important method, though fluctuations in price and availability of raw materials may, at times, curtail operations. Some production figures are given in Table 46.

TABLE 46. COMMERCIAL PRODUCTION OF BUTANOL AND ACETONE

| | *Thousands of Pounds* | | |
|---|---|---|---|
| | Total production of butanol | Production of acetone by fermentation | Production of acetone by synthesis |
| 1945 | 129,364 | 42,417 | 307,363 |
| 1946 | 126,233 | 37,436 | 298,148 |
| 1947 | 140,122 | 39,996 | 357,193 |
| 1948 | not given | 26,858 | 443,750 |
| 1949 | 120,331 | 24,825 | 388,139 |
| 1950 | 147,326 | not available | 481,668 |

Source: U. S. Tariff Commission Reports.[78]

## USES

The largest single use for *n*-butyl alcohol is the manufacture of its esters for use in the protective-coatings industry. Other important uses are in brake fluids, antibiotic recovery, urea-formaldehyde resin, and in amines for gasoline additives. Uses have been detailed by Porter[57] and by Prescott and Dunn.[58]

## BIBLIOGRAPHY

1. Arroyo, R., U. S. Patent 2,113,471 (1938).
2. Arroyo, R., U. S. Patent 2,113,472 (1938).
3. Arzberger, C. F., U. S. Patent 2,139,108 (1938).
4. Beesch, S. C., U. S. Patent 2,439,791 (1948).

4a. Beesch, S. C., *Ind. Eng. Chem.*, **44**, 1677 (1952).
5. Beesch, S. C., and D. A. Legg, U. S. Patent 2,420,998 (1947).
6. *Bergey's Manual of Determinative Bacteriology*, 6th ed., Baltimore, Williams and Wilkins, 1948.
7. Carnarius, E. H., U. S. Patent 2,198,104 (1940).
8. Carnarius, E. H., U. S. Patent 2,423, 580 (1947).
9. Carnarius, E. H., and W. N. McCutchan, U. S. Patent 2,139,111 (1938).
10. Christensen, L. M., U. S. Patent 2,182,550 (1939).
11. Christensen, L. M., and E. I. Fulmer, *Ind. Eng. Chem., Anal. Ed.*, **7**, 180 (1935).
12. Cohen, G. N., and G. Cohen-Bazire, *Ann. inst. Pasteur*, **77**, 729 (1949).
13. Cohen, G. N., and G. Cohen-Bazire, *Ann. inst. Pasteur*, **78**, 644 (1950).
14. Cohen-Bazire, G., and G. N. Cohen, *Ann. inst. Pasteur*, **77**, 718 (1949).
15. Davies, R., *Biochem. J.*, **36**, 582 (1942).
16. Davies, R., and M. Stephenson, *Biochem. J.*, **35**, 1320 (1941).
17. Frey, A., H. Glück and H. Oehme, U. S. Patent 2,166,047 (1939).
18. Gabriel, C. L., *Ind. Eng. Chem.*, **20**, 1063 (1928).
19. Gabriel, C. L., and F. M. Crawford, *Ind. Eng. Chem.*, **22**, 1163 (1930).
20. Gavronsky, J. O., British Patent 571,630 (1945).
21. Goodwin, L. F., *J. Am. Chem. Soc.*, **42**, 39 (1920).
22. Hall, H. E., U. S. Patent 2,147,487 (1939).
23. Hildebrandt, F. M., and N. M. Erb, U. S. Patent 2,169,246 (1939).
24. Jean, J. W., U. S. Patent 2,182,989 (1939).
25. Johnson, M. J., *Ind. Eng. Chem., Anal. Ed.*, **4**, 20 (1932).
26. Kawano, Y., *Hakko Kogaku Zasshi (J. Fermentation Technol.)*, **25**, 157 (1947).
27. Kelly, F. C., *One Thing Leads to Another*, Boston and New York, Houghton Mifflin, 1936.
28. Koepsell, H. J., and M. J. Johnson, *J. Biol. Chem.*, **145**, 379 (1942).
29. Lampen, J. O., and W. H. Peterson, *Arch. Biochem.*, **2**, 443 (1943).
30. Lardy, H. A., and J. A. Zeigler, *J. Biol. Chem.*, **159**, 343 (1945).
31. Lee, S. B., *Ind. Eng. Chem.*, **41**, 1868 (1949); **42**, 1672 (1950).
32. Legg, D. A., U. S. Patent 1,668,814 (1928).
33. Legg, D. A., U. S. Patent 2,223,788 (1940).

34. Legg, D. A., and H. R. Stiles, U. S. Patent 2,122,884 (1938).
35. Legg, D. A., and N. R. Tarvin, U. S. Patent 2,107,261 (1938).
36. Legg, D. A., and N. R. Tarvin, U. S. Patent 2,107,262 (1938).
37. Legg, D. A., and M. T. Walton, U. S. Patent 2,132,358 (1938).
38. Leonard, R. H., W. H. Peterson, and G. J. Ritter, *Ind. Eng. Chem.*, **49**, 1443 (1947).
39. Loughlin, J. F., U. S. Patent 2,219,426 (1940).
40. McCoy, E. F., U. S. Patent 2,110,109 (1938).
41. McCoy, E. F., U. S. Patent 2,398,837 (1946).
42. McCoy, E., E. B. Fred, W. H. Peterson, and E. G. Hastings, *J. Infectious Diseases*, **39**, 457 (1926).
43. McCutchan, W. N., Unpublished observations.
44. MacDonald, J. A., U. S. Patent 2,233,021 (1941).
45. Marvel, C. S., and A. E. Broderick, *J. Am. Chem. Soc.*, **47**, 3045 (1925).
46. Mickelson, M. N., A. Gregor, and D. Rogers, *Papers Mich. Acad. Sci.*, **34**, 19 (1948).
47. Miner, C. S., U. S. Patent 2,202,161 (1940).
48. Müller, J., U. S. Patent 2,123,078 (1938).
49. Müller, J., U. S. Patent 2,132,039 (1938).
50. Müller, J., U. S. Patent 2,195,629 (1940).
51. Nakahama, T., and Y. Haroda, *J. Agr. Chem. Soc. Japan*, **23**, 176 (1949).
52. Neerman, J., Private communication, 1951.
53. Owen, W. L., U. S. Patent 2,164,255 (1939).
54. Owen, W. L., *Blackstrap Molasses as Raw Material for Biochemical Industries*, New York, Russell Palmer, 1939.
55. Oxford, A. E., J. O. Lampen, and W. H. Peterson, *Biochem. J.*, **34**, 1588 (1940).
56. Peterson, W. H., and E. B. Fred, *Ind. Eng. Chem.*, **24**, 237 (1932).
57. Porter, J. R., *Bacterial Chemistry and Physiology*, New York, John Wiley, 1946.
58. Prescott, S. C., and C. G. Dunn, *Industrial Microbiology*, 2nd ed., New York, McGraw-Hill, 1949.
59. Reyes-Teodoro, R., and M. N. Mickelson, *Arch. Biochem.*, **4**, 291 (1944).
60. Rosenfeld, B., and E. Simon, *J. Biol. Chem.*, **186**, 395 (1950).
61. Rosenfeld, B., and E. Simon, *J. Biol. Chem.*, **186**, 405 (1950).
62. Rubbo, S. D., and J. M. Gillespie, *Nature*, **146**, 838 (1940).
63. Rubbo, S. D., M. Maxwell, R. A. Fairbridge, and J. M. Gillespie, *Australian J. Exp. Biol. Med. Sci.*, **19**, 185 (1941).

64. Secretary of Interior, Report for 1949, Section on Liquid Fuels from Farm Waste Residues.
65. Simon, E., *Nature,* **152**, 626 (1943).
66. Sjolander, N. O., A. F. Langlykke, and W. H. Peterson, *Ind. Eng. Chem.,* **30**, 1251 (1938).
67. Smyth, H. F., and W. L. Obold, *Industrial Microbiology,* Baltimore, Williams and Wilkens, 1930.
68. Stark, W. H., and W. J. McGhee, Abst. of Papers, 22A, 118th Meeting of Am. Chem. Soc., 1950.
69. Stiles, H. R., U. S. Patent 2,098,199 (1937).
70. Stiles, H. R., U. S. Patent 2,098,200 (1937).
71. Stiles, H. R., and L. M. Pruess, U. S. Patent 2,202,785 (1940).
72. Tarvin, N. R., U. S. Patent 2,260,126 (1941).
73. Thaysen, A. C., *J. Inst. Brewing,* **30**, 349 (1924).
74. Tsuchiya, H. M., J. M. Van Lanen, and A. F. Langlykke, U. S. Patent 2,481,263 (1949).
75. Underkofler, L. A., L. M. Christensen, and E. I. Fulmer, *Ind. Eng. Chem.,* **28**, 350 (1936).
76. Underkofler, L. A., E. I. Fulmer, and M. M. Rayman, *Ind. Eng. Chem.,* **29**, 1290 (1937).
77. Unger, E. D., *The Development and Design of a Continuous Cooker and Mashing System for Cereal Grains,* Thesis, Case School of Applied Science, 1941.
78. United States Tariff Commission Reports, 1945-1950.
79. Weizmann, C., U. S. Patent 1,315,585 (1919).
80. Weizmann, C., U. S. Patent 2,377,197 (1945).
81. Weizmann, C., and B. Rosenfeld, *Biochem. J.,* **31**, 619 (1937).
82. Wheeler, M. C., and C. D. Goodale, U. S. Patent 1,875,536 (1932).
83. Wiley, A. J., M. J. Johnson, E. F. McCoy, and W. H. Peterson, *Ind. Eng. Chem.,* **33**, 606 (1941).
84. Williams, A. E., *Chem. Products,* **12**, 441 (1949).
85. Wood, H. G., R. W. Brown, and C. H. Werkman, *Arch. Biochem.,* **6**, 243 (1945).
86. Woodruff, J. C., H. R. Stiles, and D. A. Legg, U. S. Patent 2,089,522 (1937).

# Part IV. THE FERMENTATIVE PRODUCTION OF ORGANIC ACIDS

CHAPTER 12

# LACTIC ACID

*H. H. Schopmeyer*

Lactic acid derives its name from its presence in sour milk where it was first discovered in 1790 by Scheele,[45] who allowed milk to ferment for 3 weeks, separated the protein, and added calcium hydroxide to precipitate the phosphate. The calcium lactate remained dissolved and was decomposed with oxalic acid. The filtrate was concentrated and taken up in alcohol. After concentrating the alcohol, the acid lactate was obtained as a thick sirup which could not be distilled or crystallized. From this first work, as well as subsequent work, Scheele concluded that the acid was free from acetate and that a new acid had been obtained. Pasteur, 77 years later in 1867, discovered that the souring of milk was caused by an organized ferment and described the organism which was responsible for the fermentation of sterilized milk. Lister,[31] in 1878, showed that the lactic fermentation is similar to the so-called alcoholic fermentation and is produced by microorganisms which he isolated from sour milk by dilution.

Lactic acid, or $\alpha$-hydroxy propionic acid, is widely occurring in nature, being present in many fermented substances, such as sour milk, sauerkraut, fermented mash or beer, in distilleries and breweries, in many food products, such as bread, in muscle tissue, and is one of the principal organic acids in the soil.

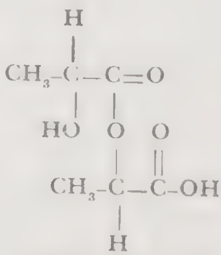
Lactic acid is a colorless to slightly yellowish sirupy liquid

with a specific gravity of 1.294 at 25°C. It is completely miscible with water, alcohol, and ether, but is insoluble in chloroform. It is somewhat hygroscopic in solutions of high concentration. It may be prepared by steam distillation under high vacuum. With ferric chloride, it gives the characteristic yellow-green color typical of $\alpha$-hydroxy acids. It yields iodoform on warming with iodine-potassium iodide solution and aqueous potassium hydroxide.

Lactic acid contains an asymmetric carbon atom and exists in the *d*- and *l*-active, and *dl*-inactive forms. The two optical isomers have been separated by fractional crystallization of pure lactic acid from mixed ethyl ether and isopropyl ether solution.[7] Much confusion exists regarding the nomenclature of the optical isomers. That which shows dextro rotation (commonly called *d*-lactic acid or sarcolactic acid) is properly designated by modern nomenclature as L-(+)-lactic acid; when pure, it has a melting point of 52.8°C This form of lactic acid is metabolized completely by the human body.[13] Salts of the dextrorotatory acid are levorotatory.

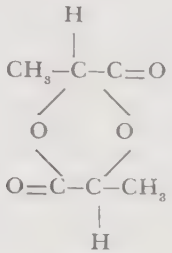
The levorotatory lactic acid, properly designated as D-(—)-lactic acid also has a melting point of 52.8°C. It is not metabolized by the animal body and is largely excreted as such. Its salts are dextrorotatory. Vigorous heating of the optically active lactic acids converts each into the corresponding optically inactive racemic form containing equal proportions of the dextro and levo modifications.

In commercial production, ordinarily only the inactive racemic form is recovered even though an active form is produced as a result of the fermentation. Traces of impurities generally present will racemize the active form.[61] When lactic acid in water solution is concentrated, it dehydrates to form the anhydride, $CH_3 \cdot CHOH \cdot CO \cdot O \cdot CO \cdot CHOH \cdot CH_3$, a viscous sirupy liquid which will readily hydrolyze to the free acid on boiling the dilute solution with acid or alkali. Further concentration to a temperature of 130° to 140°C results in the production of lactyl lactic acid,



Lactyl lactic acid

which is an amorphous viscous mass, almost completely insoluble in water, but soluble in alcohol and ether. Prolonged heating of lactic acid at 140°C and 10 mm pressure converts it to the lactide,[11]



Lactide

which can be recovered by distillation. The lactide forms white needlelike crystals with a melting point of 128°C and a boiling point of 255°C. It is very difficultly soluble in water or alcohol and easily soluble in acetone. The lactide is slowly hydrolyzed to lactic acid by prolonged boiling with water or rapidly by boiling with alkali.

Lactic acid has been produced commercially in the United States for many years, first by Charles E. Avery, at Littleton, Massachusetts, in 1881[24] in an attempt to make calcium lactate which was to be used as a substitute for cream of tartar in baking powder. This first venture was unsuccessful and the development of industrial applications in leather, textiles, food, and solvents did not start until later. Lactic acid production was likewise developed in Europe and, for many years, much of the highest grade acid used in the United States was imported from Germany. Improved processes in purifying crude acid in the United States, however, had effectively stopped this importation of German acid well before World War II.

## RAW MATERIALS FOR LACTIC ACID PRODUCTION

Industrial lactic acid is prepared by the fermentation of a number of sugars, including dextrose, maltose, lactose, and sucrose. A wide variety of raw materials could be used for lactic acid production, but the difficulties encountered in processing the fermented liquor restrict somewhat the materials actually used. It has been generally conceded that it is more advantageous to start with a considerably refined substance than to attempt much purification

of fermentor liquor or crude acid. Therefore, dextrose has probably been more widely used than any other substance for lactic acid production. A report[26a] has recently been published which reviews in considerable detail all phases of the production of lactic acid from dextrose. Considerable acid is made from molasses and whey and some from hydrol.

In the fermentation of dextrose to lactic acid, nutrients must be added to supply nitrogen for the fermentation. The amount of these added nutrients is kept at a minimum so that the fermentation is not unduly contaminated with substances which are difficult to remove in the later purification. A typical example is described in the patent of Walsh and Needle,[57] in which starch-conversion liquor, containing a substantial amount of dextrose, is fermented with *Lactobacillus delbruckii*, using nondenatured milk as an added nutrient. A similar patent[33] involves the use of grain-protein derived from distillers' grains, etc., as nutrient for lactic acid fermentation. The inclusion of small quantities of unheated malt sprouts greatly accelerates the fermentation. The malt sprouts contain a heat-labile growth factor for *L. delbruckii*.[41] This factor appears to be stable to heating for 10 minutes at 65°C, but exposure for longer periods at higher temperatures is destructive to it. It has been well established that lactic acid bacteria require certain supplementary factors for growth.[39,51] The precise requirements vary with the organisms, but for the most part include the vitamins essential to animals.[50] It is thus apparent that carbohydrate media for fermentations, containing sugar and inorganic salts, must be supplemented by nitrogenous substances from a natural source which will contain these growth-promoting supplements.

Whey is considered a potentially important raw material for lactic acid fermentation, and it was estimated[47] that, based on an annual milk production of 100 billion lb, about 2.7 billion lb of lactose could be made available for fermentation. Lactic acid has been produced by one company[9] in limited quantities from whey for a number of years. Apparently, cost of collecting the whey and general processing problems have prevented this source from assuming greater importance.

Xylose and arabinose have been studied as possible raw materials in lactic acid production, using a pentose fermenting

organism, *Lactobacillus pentoaceticus*.[21] As starting materials, corncobs, corn stalks, cottonseed hulls, straw and similar materials could be used. Based on laboratory data, corncobs, when hydrolyzed and fermented by *L. pentoaceticus,* produced 300 lb of acetic acid and 320 lb of lactic acid per ton of cobs.[20]

Fries[23] obtained a patent on the utilization of sulfite waste liquor for the lactic acid fermentation. The liquor was prepared for fermentation by pretreatment with lime, filtration, and adjustment of the pH at 6.3 to 6.7. The temperature was adjusted to 30° to 40°C, nutrients, such as malt sprouts or corn steep liquor, were added, and the liquor was fermented with various lactobacilli, a strain of *L. pentosus* giving best results. In a more recent patent of Ekelund,[15] the carbohydrate was extracted from sulfite waste liquor by "sorping" on lactic acid bacteria prior to lactic fermentation by them.

The possible use of cull fruit was suggested by Nolte and Van Loescke[37] in describing a fermentation which they carried out at 50°C for 6 to 8 days, with the lactic acid bacilli normally present in grapefruit. They obtained a recovery of 6% calcium lactate and 7.1% of 50% lactic acid, based on the weight of the juice used. On this basis, a ton of grapefruit is equivalent to about 40 lb of dextrose as a raw material for the production of lactic acid.

Direct fermentation of starch or starchy substances for production of lactic acid has received some attention. For example, Kitahara and Ishida[28a] studied simple fermentation of starch to lactic acid with *Lactobacillus thermophilus.* The organism was cultured at 50°C for 5 to 10 days on pastes of cereal and tuber materials. The starch was liquefied within 1 day. The lactic acid yields were over 80%, except from corn and commercial starch. The addition of sodium chloride to the cereal paste produced better fermentation. The maximum lactic acid yield of 97% was obtained from barley-bouillon paste, containing 1% sodium chloride and cultured 5 days.

Other raw materials for lactic acid fermentation mentioned from time to time include Jerusalem artichokes,[2] potatoes,[12,54] molasses,[40] and beet juice.[6]

Both the spent beer stillage from alcoholic fermentation of grain and the steep water in the corn wet-milling process contain

considerable quantities of lactic acid. The stillage contains 6 to 8% of lactic acid on a dry-substance basis and the corn steep water as high as 20% on a dry-substance basis. This lactic acid might be recovered if a suitable extraction technique could be developed. This has been attempted in investigations by Laszloffy[29] in which distillery slops were acidified, evaporated in vacuum, and treated with ether to remove the fats and organic acids. Corn steep water might be purified by some similar process or by crystallizing magnesium lactate from the steep water solution on concentrating and allowing to stand. The magnesium is apparently derived from the corn during the steeping process.

Mention should be made of other possible methods for the manufacture of lactic acid, notably, degradation of carbohydrates as described by Braun,[8] in which a suitable carbohydrate, such as sucrose, is heated with a strong alkali, such as calcium hydroxide, at a high temperature and pressure to produce lactic acid. Another interesting method for the synthesis of lactic acid is the heating of carbon monoxide and acetaldehyde in the presence of sulfuric acid at 130° to 200°C under 900 atm pressure.[32] In addition to these novel methods for preparing lactic acid, there are several classical methods such as: (1) from acetaldehyde and hydrocyanic acid; (2) by action of nitrous acid on alanine; (3) by oxidation of propylene glycol, and others.

## LACTIC ACID PRODUCING ORGANISMS AND THE MECHANISM OF THEIR REACTIONS

Many bacteria are capable of producing lactic acid, but frequently other products, notably acetic acid, are also produced in considerable amounts. Through the work of Kayser,[28] it has become customary to subdivide the lactic acid bacteria into two main groups: (1) those organisms, described as the true lactic acid bacteria, which produce substantially only lactic acid, and (2) those bacteria which, in addition to lactic acid, produce volatile acids in considerable amounts. These two groups are also referred to as homofermentative and heterofermentative.

The homofermentative or true lactic acid bacteria produce lactic acid almost exclusively. Therefore, the over-all chemical

equation for the production of lactic acid by these organisms may be very simply given as:

$$C_6H_{12}O_6 \longrightarrow 2\,CH_3{\bullet}CHOH{\bullet}COOH$$

or in the case of a disaccharide:

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow 4\,CH_3{\bullet}CHOH{\bullet}COOH$$

The initial stages of homofermentative lactic acid fermentation are considered to be similar to those of the ethanol fermentation (see Figure 1, Chapter 2) down to the glyceraldehyde phosphate stage. The reactions which occur between the triose and lactic acid have not been fully proved and two possible mechanisms have been suggested. According to Neuberg and Gore,[35] the final intermediate is methylglyoxal, which, in turn, is converted to lactic acid by glyoxalase and its coenzyme, glutathione. According to this scheme the reaction might be:

$$\underset{\text{Dextrose}}{C_6H_{12}O_6} \longrightarrow \underset{\text{Glyceraldehyde}}{CH_2OH{\bullet}CHOH{\bullet}CHO} \longrightarrow$$

$$\underset{\text{Methylglyoxal}}{CH_3CO{\bullet}CHO} \xrightarrow{\text{glyoxalase}} \underset{\text{Lactic acid}}{CH_3{\bullet}CHOH{\bullet}COOH}$$

The second proposed mechanism involves the dissimilation of dextrose to pyruvic acid in the same manner as in the alcoholic fermentation. The pyruvic acid is then reduced to lactic acid:

$$\underset{\text{Pyruvic acid}}{CH_3{\bullet}CO{\bullet}COOH} + 2\,H \longrightarrow \underset{\text{Lactic acid}}{CH_3{\bullet}CHOH{\bullet}COOH}$$

Bernhauer[4] cites experimental evidence which suggests that either or both of these mechanisms may be operative during the homofermentative lactic acid fermentation.

The mechanism for the fermentation of dextrose by the heterofermentative lactic acid producing bacteria is much more complicated. Nelson and Werkman[34] studied the fermentation of dextrose with several heterofermentative lactic acid bacteria, including *L. acidophil-aerogenes* and *L. lycopersici* and, from experimental data obtained, proposed a tentative scheme for the dissimilation of dextrose by heterofermentative bacteria. Their scheme is as follows:

$C_6H_{12}O_6$
Dextrose
↓
Hexose phosphate
↓

$$\begin{array}{lcl} CHO & & CH_2OH \\ | & & | \\ CHOH & +2H \longrightarrow & CHOH \\ | & & | \\ CH_2OH & & CH_2OH \\ \text{glyceraldehyde} & & \text{Glycerol} \end{array}$$

↓
$C_3H_6O_3$
Hypothetical three-carbon compound

$$\begin{array}{ll} \downarrow & \downarrow \\ \text{Acetaldehyde} + CO_2 + H_2 & \text{Lactic acid} \\ \Big\downarrow + 2H & \downarrow \\ \text{Ethyl alcohol} & \text{Pyruvic acid} + 2H \\ & \Big\downarrow H_2O \\ & \text{Acetic acid} + CO_2 + 2H \end{array}$$

The true lactic acid bacteria are most commonly used in industrial fermentations and are generally long, slender, Gram-positive rods, nonmotile, nonsporulating, and produce very little gas which is chiefly carbon dioxide. They are, for the most part, somewhat microaerophilic. Of the organisms of this classification *Lactobacillus delbruckii* is probably the most widely used. When freshly isolated, this organism usually ferments maltose well but not dextrose. It ferments dextrose vigorously when properly acclimatized, the speed of fermentation being largely determined by the type and quality of the nutrients added. The acclimatization is sometimes not so easy as one might expect from reading the scientific literature. It has been claimed that an active dextrose-fermenting culture is obtained by the following procedure. *L. delbruckii* is brought to vigorous growth in maltose solution, then the organism is gradually accustomed to dextrose by daily additions of portions of dextrose solution.[22] Some strains of *L. delbruckii* respond to such treatment while others do not. It was found that some organisms that had been isolated for several years could not be developed to ferment dextrose satisfactorily.

In fermenting whey to lactic acid, *L. bulgaricus*[9] is commonly used, since it is capable of fermenting lactose while *L. delbruckii* is

not. As already mentioned, the fermentation of wood sugar has been carried out experimentally by several investigators,[1,20] using *L. pentoaceticus.* This organism exists as short, blunt rods; it is gram-positive and microaerophilic. It produces lactic acid, ethyl alcohol, carbon dioxide, and small quantities of acetic acid from aldohexoses, while from xylose, much more acetic acid is produced. The lactic acid produced by this organism is not optically active.

Another organism was reported by Werkman and Anderson[62] to be capable of producing dextro-lactic acid. This was described as an aerobic, sporulating, gram-positive, motile, catalase-positive, nitrate-reducing bacillus with an optimum temperature of 46° to 50°C. Yields of more than 95% dextro-lactic acid were reported in 5 days' fermentation of 15% dextrose solution. Acetylmethylcarbinol and 2,3-butylene glycol were formed in small amounts. A colorless acid was prepared directly from this medium.

The writer has isolated a similar organism several times from malt sprouts by natural selection. It was generally found as a granular, gram-positive, spore-forming rod. It fermented dextrose very vigorously with good yields.

The production of lactic acid by mold fermentation has been investigated at length by several workers. Ward, Lockwood, Tabenkin, and Wells[58] reported a rapid fermentation process for dextro-lactic acid using *Rhizopus oryzae* in submerged culture. They were able to ferment 13% dextrose solution in a rotary aluminum fermentor under 5 psi pressure with circulating air in 30 to 35 hours, with a 70 to 75% yield of dextro-lactic acid. A carbon balance showed that a yield of 70 to 75% lactic acid was produced with a small amount of ethanol and an unidentified substance equivalent to 7.4% of the sugar consumed. The medium contained dextrose, magnesium sulfate, potassium dihydrogen phosphate, calcium carbonate, and urea. It was found that using urea as a source of nitrogen, rather than cruder nitrogenous substances, such as grain residue used in bacterial fermentations producing lactic acid, resulted in a finished product which was readily purified to a high grade acid.

Bernhauer, Rauch, and Miksch[5] recently made an extensive investigation of the production of lactic acid by *Rhizopus* species in submerged aerated cultures. Best results were obtained with a strain of *R. oryzae.* Yields of 70 to 80% of lactic acid were attained in about 7 days from dextrose in the presence of calcium carbonate,

but from starch, the yields were only 45 to 48%. A preliminary investigation of the effect of trace elements was made. The best nitrogen sources were urea and ammonium sulfate. The use of submerged mycelium or partially germinated spores, instead of a spore suspension, markedly accelerated the fermentation.

## MAINTENANCE OF CULTURES

Cultures of lactic acid bacteria can be maintained on dextrose nutrient agar or dextrose-peptone media containing an excess of calcium carbonate. Frequent transfers are necessary to maintain viability. In investigating other methods of storage, it was found that the organisms had a considerably longer life when transferred into sterile 5% corn mash containing an excess of calcium carbonate. Under these conditions, when cultures were stored in a refrigerator, the organisms remained viable for several months. Attempts to prepare dry cultures on soil were entirely unsuccessful.

## PLANT FERMENTATION

In developing the culture to plant stage, it is customary to transfer from test tubes to 1,000-ml flasks and then to 5-gal bottles. Throughout these development stages, the cultures are not agitated, but a large excess of calcium carbonate is maintained to neutralize the acid formed. Cultures in the plant are propagated through small culture tanks to larger tanks and to fermentors, maintaining an inoculation ratio of about 5%. An incubation period of 16 to 18 hours is ordinarily sufficient to obtain a vigorous and abundant development of organisms. The optimum temperature for fermentation with commercial types of lactic acid organisms is about 45°C and this temperature must be assiduously maintained. A temperature this high is outside the growth range for most contaminating organisms, so that contamination is generally not a very serious problem. However, if the temperature drops somewhat during fermentation, conditions become very favorable for the butyric acid fermentation and butyric organisms may develop and seriously impair the quality of the finished product.

Likewise butyric acid contamination sometimes becomes a problem when the lactic acid fermentation is nearly finished and the activity of the lactic organisms has greatly diminished. Apparently the butyric acid organisms metabolize the lactate ion and

in instances of severe butyric acid contamination, the increase in butyrate-ion content has been found to be accompanied by a decrease in lactic ion.

The commercial fermentation is ordinarily carried out in about 12% (w/v) sugar solution. A sugar concentration much higher than this will result in calcium lactate crystallizing in the later stages of the fermentation. The medium will then become unduly heavy and difficult to handle and the proper completion of the fermentation will be retarded.

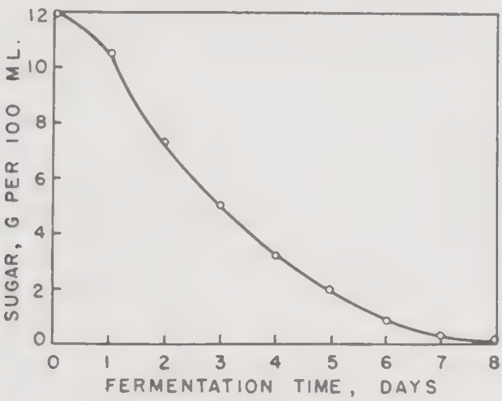



FIGURE 61. *Change of Sugar Concentration during Lactic Acid Fermentation*

The fermentation progresses rapidly in the initial stages, with the concentration of sugar frequently dropping as much as 3 to 4 g per 100 ml in 24 hours, as shown in Figure 61. The rate of fermentation decreases as it nears completion and when most of the sugar has been consumed, the residual sugar may disappear as slowly as 0.1 to 0.2 g per 100 ml per day. The fermentation time will vary from batch to batch and will range from about 5 to 10 days. An excess of calcium carbonate is used and the fermenting liquor is agitated vigorously and constantly during the fermentation in order to keep the neutralizing agent in suspension to maintain the proper pH. Most *L. delbruckii* type cultures operate best in the pH range of 5.5 to 6.5; below pH 5.5, in the very lean medium used in commercial production, the fermentation is greatly retarded. However, in a grain-mash medium of a much higher protein concentration, the organisms are capable of fermenting vigorously at a considerably lower pH.

It is of extreme importance to carry the fermentation to as near completion as possible, since residual carbohydrates will seriously interfere with the later processing of the acid liquor. Residual carbohydrates should be reduced to 0.1% or less as determined by copper-reducing methods. It has been found possible to greatly accelerate the rate of fermentation by better control of pH by continuous neutralization with calcium hydroxide, controlling the pH between 6.3 to 6.5 automatically. Under these conditions, 12 to 13% sugar solutions were fermented in 72 hours of actual fermenting time. Laboratory studies on lactic fermentation of dextrose under closely controlled conditions[17] showed that the rate of production of lactic acid was greatest at pH 5.7. This rate was also greatly increased by increasing the concentration of accessory growth factors in the medium.

Fermentations are customarily carried out batchwise in commercial operations, but Whittier and Rogers[63] developed a laboratory method for continuous fermentation of whey which was later adapted to commercial operation. While such a method is mechanically workable, it is quite likely that difficulties would be encountered in its steady operation because of the extremely variable rate of fermentation by the organisms in the very lean medium in which they are grown. Since it is important to remove substantially all the sugar prior to processing the fermented liquor, it is doubtful if continuous fermentation will have wide application. Further, the savings that can be realized from continuous fermentation are largely from economies in labor and comparatively cheap fermenting equipment and these advantages could be quickly offset by difficulties encountered in processing liquor which had not been properly fermented.

After completion of fermentation, the fermented liquor is treated with lime to a pH of 10 to 11, then heated to boiling to kill the organisms present, coagulate the proteinaceous material to allow better filtering in removing the excess calcium carbonate and proteinaceous material, and to help decompose any remaining unfermented sugar.

## GENERAL EQUIPMENT

Lactic acid is quite corrosive and, except in the fermentation stage, corrosion-resistant material must be used. Culture tanks and

fermentors can be made of wood provided they are sufficiently heavy; the calcium lactate soaks through the wood very badly, opens the pores and deteriorates the wood. Equipment for handling acid must be of highly resistant material. For mechanical filters, dilute-acid tanks, etc., rubber-clad steel is satisfactory provided the temperature is not too high; pumps, pipe lines, and evaporating equipment are subject to extreme corrosion and difficulties have been encountered with all corrosion-resistant materials. Nickel and monel, copper, bronze, etc., are not considered sufficiently resistant. Silver and tantalum are resistant but too costly. High-molybdate stainless steel, such as 316SS has good corrosion resistance, but much difficulty has been encountered in welds not properly annealed.

Many of the resinous materials which normally have high acid resistance are solubilized or softened by lactic acid, particularly when it is warm and concentrated. The ceramic materials, porcelain, glass piping, glass or enamel-clad materials have only limited application because of brittleness, difficulty of fabrication, and poor heat transfer. In the case of enamel-clad materials, the shock of sharp temperature changes soon deteriorates the coating.

## GRADES OF LACTIC ACID: FLOWCHARTS AND SPECIFICATIONS

Lactic acid is sold in four grades and several concentrations in each grade.

The crude acid or technical grade which is of rather indefinite color is sold in 22, 44, 50, 66, 80%, and other concentrations. The regular edible grade, a light, straw-colored product, is sold customarily in 50 to 80% concentrations; the plastic grade, a colorless product, is sold in 50 to 80% concentrations, while the U.S.P. grade is sold at 85% concentration. Typical analyses of these various grades of acids are shown in Table 47.

The technical grade of acid, not requiring a high degree of purity, can be made directly from fermented sugar liquor, etc., by direct decomposition of the calcium lactate with sulfuric acid, filtering off the calcium sulfate, and concentrating.[42] Since the initial liquor is quite dilute, containing about 9 to 10% calcium lactate after the wash water from fermentors, filters, etc., is added, a large amount of calcium sulfate will precipitate on concentrating,

TABLE 47. GRADES OF LACTIC ACID

| | Technical | Edible | Plastic | U.S.P. |
|---|---|---|---|---|
| Total acidity | 44% | 50% | 50% | 85% |
| Free acidity | 40–42% | 46–48% | 47–49% | 76–78% |
| Volatile acids | 1–2% | 1–2% | 1–2% | 2-3% |
| Ash | 0.6–0.7% | 0.4–0.5% | 0.005–0.01% | 0.05–0.1% |
| Carbonizable organic matter | present | present | none | none |
| Sulfates | present | trace | none | none |
| Chlorides | present | present | none | none |
| Color | yellow to brown | slight straw yellow | colorless | colorless |
| Iron | present | less than 3 ppm. | trace | trace |
| Copper | present | trace | trace | trace |

which will require removal by filtration at about 30 to 35% concentration. Since such crude technical acid contains many of the impurities present in the starting material, the quality and nature of the starting material will largely determine the quality of the technical acid made from it.

The regular edible grade of acid requires considerably more refining than the technical grade either in the raw material or in the finished product and the source of raw material probably determines the choice of refining method. A flow diagram of the process for edible lactic acid is shown in Figure 62. In making edible grade of acid from corn sugar, it is customary to refine the sugar to a high degree of purity, ferment it in a very-low-protein medium until the sugars are completely removed, decompose the calcium lactate liquor with sulfuric acid, bleach the light liquor, concentrate at 25 to 30%, bleach again, and finally concentrate at 55 to 60% and bleach for a third time. Benefits may also sometimes be noted in bleaching the calcium lactate liquor before decomposing. After final concentration, the liquor may contain considerable iron and copper, etc., from the processing equipment which imparts a very objectionable green-yellow color to the liquor and must be removed by addition of sodium ferrocyanide to form the corresponding iron and copper salts which precipitate and can be removed. A somewhat better grade of acid can be made by crystallizing the calcium lactate and purifying it by filtration or centrifugation before decomposing. This method is not without difficulties, since the calcium lactate tends to crystallize in agglomerates of needles which

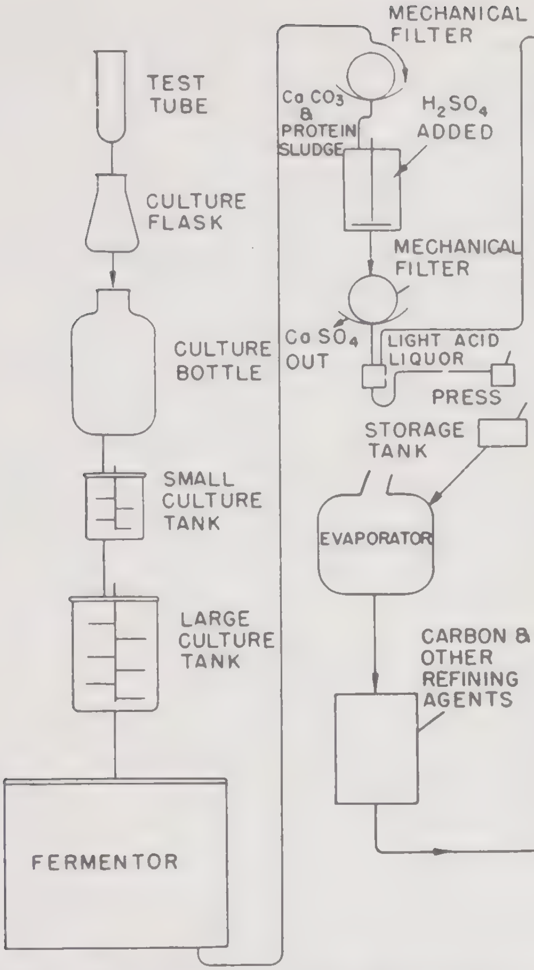



FIGURE 62. *Flow Diagram of the Production of Edible-Grade Lactic Acid*

occlude considerable mother liquor and are difficult to wash and also, the calcium lactate is of limited solubility under crystallizing conditions and the yield is comparatively small. Acid from this process will be low in unfermented carbohydrates and their decomposition products, but will contain ash, particularly calcium sulfate, which can be removed, to some extent, by chemical precipitation.

Several processes have been developed in the past few years for production of higher grades of acid suitable for plastics, certain

edible uses, and for U.S.P. quality. Perhaps the oldest of these improved methods is that of steam distillation under high vacuum.[19,30,32] This method is not particularly efficient, because the recovery of high quality acid is comparatively low. Much acid decomposes during distillation and the residue, after distillation, is a black tarry mass which is largely anhydride, etc., and has a limited recovery value. The yield and quality of the distillate are largely dependent on the quality of the acid supplied to the still. If considerable carbohydrate material remains after fermentation and preliminary processing, the distilled acid is very likely to show the presence of carbonizable organic substances by the brown ring test[3] due to their decomposition and subsequent distillation, probably chiefly as hydroxymethylfurfural. Several methods, relying chiefly on oxidizing agents, for purifying acid containing such substances have been patented. An example is that of Haag,[25] in which a small amount of nitric acid is added to the lactic acid and the mixture heated.

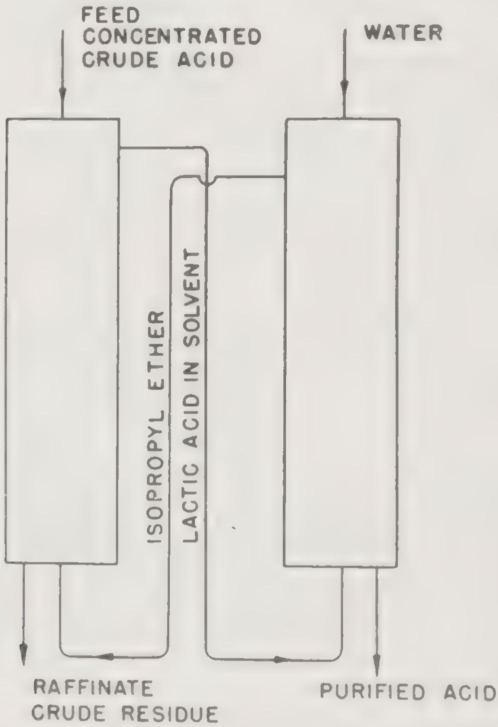



FIGURE 63. *Isopropyl Ether Extraction System for Purifying Lactic Acid*

Another method for purifying lactic acid has been patented by Jenemann,[27] in which the crude acid is extracted continuously in a counter-current system (see Figure 63), using isopropyl ether as the solvent. The acid is recovered from the solvent by counter-current washing of the solvent in the second column of the system with water. The lactic acid is recovered by evaporating the aqueous solution. Other solvents can be used, such as diethyl ether, butyl alcohol, etc., but partition coefficients make them less suitable than isopropyl ether. The acid produced by this method is substantially free from ash, but will contain traces of impurities from the carbohydrates used in the fermentations and must be further refined by carbon bleaching, oxidation, and by other means necessary to make it a satisfactory product.

A third method for making high-quality acid is through the methyl ester and this is probably the most practicable of all. This process consists of preparing the methyl ester of lactic acid (see Figure 64), distilling it free from impurities, and hydrolyzing the ester by boiling in dilute water solution. The regenerated lactic acid is recovered by evaporation while the methyl alcohol is distilled off. The process was originally proposed by Hillringhaus and Heilman,[26] in 1905 and by Byk,[10] in 1912. Several recent modifications of the method have been reported. Smith and Claborn[48] prepared pure water-white acid, starting with dry calcium lactate dissolved in a large excess of methyl alcohol. Sulfuric acid was added and the calcium sulfate was removed by filtration. The excess methyl alcohol was distilled at atmospheric pressure and the water and methyl lactate were distilled under vacuum. The mixture of methyl lactate and water was further diluted, boiled to hydrolyze the ester, and the acid was finally concentrated. A yield of 85% pure acid was reported.

Wenker[59] patented a process for esterifying crude 70 to 85% lactic acid, distilling off the ester with excess alcohol, and subsequently hydrolyzing the ester. The alcohol was fractionated continuously from the ester and excess alcohol mixture, and also from the hydrolysis mixture, and was returned continuously to the acid under treatment.

Schopmeyer and Arnold[46] patented a process for continuous esterification, distillation, and hydrolysis for the purification of lactic acid, using methyl alcohol.

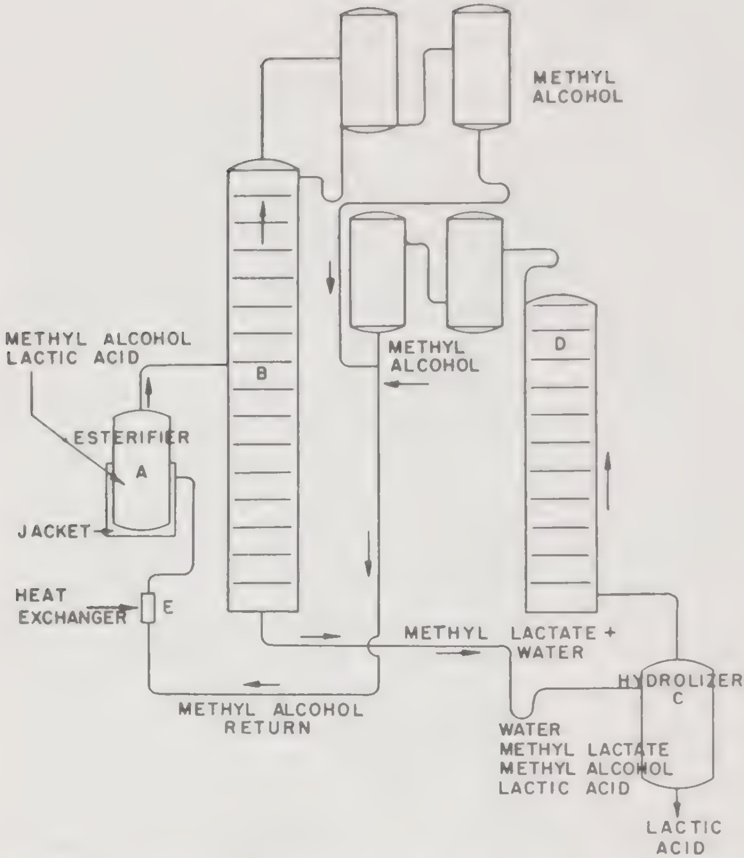



FIGURE 64. *Continuous Process for the Ester Method of Refining Lactic Acid*

Filachione and Fisher[16] studied various details of the method, particularly the ratio of alcohol to acid, effect of the concentration of catalyst, and other factors. They showed that when methanol vapors are passed through an 82% lactic acid solution, approximately 9 moles of methanol are required per mole of acid volatilized and that more methyl alcohol is required to volatilize the acid from dilute than from concentrated solutions.

Commercial production of lactic acid has been carried out on a continuous unit, in accordance with the flowsheet of Figure 64. In this system, crude lactic acid is fed into esterifying kettle A where it is vaporized with methyl alcohol through heat exchanger E. The vapors from A are passed into column B where methyl alcohol is taken off the top and methyl lactate and water off the bottom. The

methyl lactate is hydrolyzed in kettle C and methyl alcohol from the hydrolysis is recovered through column D and returned to esterifying kettle A. The acid obtained by this process is free from ash and low in other impurities and meets all specifications for U.S.P. and plastic grades. In this operation, serious trouble is again encountered with corrosion; there is sufficient action of lactic acid on stainless steel to dissolve enough iron to contaminate the product. Ceramic equipment does not withstand the prolonged action of strong acid and frequent temperature changes, particularly in the esterifying and hydrolyzing vessels. Gaskets also are difficult to maintain with so many components in the system.

## ANALYTICAL METHODS

Analytical methods for the control of the process and for checking on the finished product are, for the most part, conventional methods and do not require detailed elaboration. Analytical control used on fermentor liquor consists generally of pH measurements, determination of unfermented sugars by Munsen-Walker, or any other conventional method, and titration of the calcium lactate in the clarified fermentor liquor with sodium carbonate in the presence of thymol blue indicator.

Since all lactic acid contains some anhydride, determination of free acid and anhydride is of some interest. It is generally considered sufficient to determine free acid by titrating a tared portion of diluted acid with standard sodium hydroxide solution in the presence of phenolphthalein indicator. In order to determine the amount of anhydride present, an excess of standard sodium hydroxide is added after titrating the free acid and the alkaline solution refluxed for a few minutes. It is then back titrated with standard sulfuric acid to determine the unused alkali. From the two titrations, the amount of free acid and the amount of anhydride can be calculated.

In the plastic and U.S.P. grades of acid, the presence of traces of carbohydrates or carbohydrate-derived impurities is generally determined by the brown-ring test. This consists of placing a small amount of the acid to be tested in a test tube, inclining it, and running concentrated sulfuric acid down the inclined side of the test tube underneath the lactic acid solution. The tube is allowed to stand for a time to see whether a brown ring forms at the inter-

face of the two liquids, which indicates the presence of carbohydrate, or carbohydrate-derived impurities.

Inorganic substances, which are common impurities in lactic acid, are determined in the usual way; the chlorides with silver nitrate, sulfates with barium chloride, and ash by igniting a 5 or 10 g portion in a platinum crucible. Volatile acids are determined by distilling an aliquot portion from a diluted sample and titrating the distillate with sodium hydroxide in the presence of phenolphthalein. The volatile acid is calculated as acetic.

In the analysis of calcium lactate powder or crystals, the purity or the percentage of calcium lactate can be determined by dissolving in water and titrating hot with sodium carbonate in the presence of thymol-blue indicator. Volatile acids in calcium lactate can be determined by distilling an aliquot portion from the calcium lactate solution with sulfuric acid and titrating the acid in the distillate with sodium hydroxide, in the presence of phenolphthalein indicator.

In order to determine whether or not butyric acid is present in the distillate, portions of the distillate can be extracted with isopropyl ether and titrated,[60] and from partition coefficient data, the percentage of butyric acid in the volatile acids can be calculated.

## YIELDS

The yield of lactic acid from fermentation is 93 to 94% of the carbohydrates fermented, and this value does not seem to vary appreciably with the various commercial strains of organisms used. In contrast, the yield of finished acid will vary widely, depending on the method of processing. In the production of technical-grade acid, where minimum filtering and bleaching are required, the yields are 85 to 90% of the carbohydrates fermented. Losses are largely caused by incomplete washing of filter cake, by entrainment of lactic and volatile acids during evaporation, and by miscellaneous minor process losses.

In the production of edible grade of acid by direct refining of the crude liquor, somewhat lower yields are obtained, generally about 80%, since more steps and more handling are involved in the refining operations.

In the production of plastic- or U.S.P-grade acid by distillation of technical-grade acid, the yields are quite variable, depending on

the quality of the crude acid used. If it is of reasonably good quality, with but little unfermented carbohydrate materials, a yield of 70 to 75% of high-quality acid might be obtained with 10 to 15% still residue, consisting of lactide and various lactic acid polymers, with 10 to 15% unaccounted for. This loss is probably traceable to decomposition products during the distillation. The still residue obtained is very dark and of little value except for dark-grade technical acid.

In the production of plastic or U.S.P. grades of acid through the methyl ester process, using technical acid as the starting material, a 90 to 95% recovery of high-grade acid can be obtained, since there is little loss in processing and the still residue can be kept at a minimum.

## ECONOMICS OF LACTIC ACID PRODUCTION

The total volume of lactic acid produced increased greatly during the years of World War II and in trade circles has been estimated at about 10,000,000 lb annually. The United States Department of Commerce data given in Table 48 show total production considerably lower than this. According to these data, the volumes of domestic production were increasing while imports were decreasing.

Because of the very high price of resistant equipment and the large number of processing steps, the cost of the equipment in lactic acid manufacture is an extremely important item in the over-all cost of lactic acid production. The costs of raw materials and chemicals are comparatively low, the approximate figures being as follows:

| | |
|---|---|
| Dextrose at $7.00 per cwt | $9.50 |
| Calcium carbonate at $10.00 per ton | 0.50 |
| Sulfuric acid, (62%) at 67¢ per cwt | 0.70 |
| Bleaching and refining agents | 0.70 |
| Lime and miscellaneous chemicals | 0.10 |
| Total cost of raw materials and chemicals per 100 lb of 100% lactic acid | $11.50 |

Other possible raw materials, such as crude corn sugar, hydrol, molasses, whey, or sugar from whole-grain mashes would generally be considerably cheaper than dextrose, but against their lower cost must be weighed possible lower yields and added processing costs for refining the acid made from these crude materials, or the cost of different methods of refining.

TABLE 48. PRODUCTION AND IMPORTS OF LACTIC ACID

| Year | *Production, lb × 10³* Technical | Edible and medical | *Imports, lb* Total all grades |
|---|---|---|---|
| 1910 | —— | —— | 185,935 |
| 1911 | —— | —— | 201,311 |
| 1912 | —— | —— | 335,335 |
| 1913 | —— | —— | 158,754 |
| 1914 | —— | —— | 242,134 |
| 1915 | —— | —— | 143,291 |
| 1916 | —— | —— | 17,845 |
| 1917 | —— | —— | 100 |
| 1918 | —— | —— | 330 |
| 1919 | —— | —— | 11,033 |
| 1920 | —— | —— | 133,756 |
| 1921 | —— | —— | 593,315 |
| 1922 | —— | —— | 385,840 |
| 1923 | —— | —— | 184,937 |
| 1924 | —— | —— | 290,684 |
| 1925 | —— | —— | 588,958 |
| 1926 | —— | —— | 202,477 |
| 1927 | —— | —— | 483,150 |
| 1928 | —— | —— | 377,270 |
| 1929 | —— | —— | 404,315 |
| 1930 | —— | —— | 266,726 |
| 1931 | —— | —— | 488,848 |
| 1932 | —— | —— | 477,441 |
| 1933 | —— | —— | 547,230 |
| 1934 | —— | —— | 497,810 |
| 1935 | —— | —— | 116,592 |
| 1936 | —— | —— | 80,401 |
| 1937 | —— | 927 | 62,223 |
| 1938 | —— | 1,292 | 2,424 |
| 1939 | 1,530 | 1,609 | 1,650 |
| 1940 | 1,492 | 1,672 | 1,519 |
| 1941 | 4,243 | 3,242 | 438 |
| 1942 | 3,124 | 2,931 | not available |
| 1943 | 2,324 | 2,646 | not available |
| 1944 | 4,458 | 4,161 | not available |
| 1945 | 3,467 | 4,672 | not available |
| 1946 | 2,219 | 3,824 | not available |
| 1947 | 2,526 | 3,803 | not available |
| 1948 | 2,316 | 2,676 | not available |
| 1949 | 1,700 | 2,838 | not available |

Note: Earlier figures are not available on production because less than three firms reported, no census was taken, or lactic acid was not separately enumerated.
Source: U. S. Dept. Commerce, Special Report.[55]

Low yields of the finished product, excessive cost of maintenance due to the extremely corrosive action of the acid liquor even on resistant types of equipment, high labor and other operating costs

make the price of the finished acid quite high as compared to the raw materials. Because of the high costs, lactic acid is frequently at a serious disadvantage as compared with competing acids (see Table 49).

TABLE 49. COMPARATIVE SELLING PRICES OF LACTIC ACID AND SOME COMPETING ACIDS

| | Selling price per cwt commercial product | Approximate selling price per cwt 100% acid |
|---|---|---|
| Lactic acid, 44% technical | $11.00 | $25.00 |
| 50% edible | 16.00 | 32.00 |
| 50% plastic | 19.75 | 39.50 |
| 85% U.S.P. | 69.50 | 81.00 |
| Acetic acid, 50% | 7.90 | 15.80 |
| glacial | 13.50 | 13.50 |
| Propionic acid | 18.00 | 18.00 |
| Citric acid | 27.00 | 27.00 |
| Tartaric acid | 37.50 | 37.50 |
| Phosphoric acid, 50% food grade | 5.25 | 10.50 |

Source: *Oil, Paint and Drug Reporter,* **155**, No. 1, 9 (1949).

Improvements in refining methods for high quality, better design and better processing equipment, as well as larger and more efficient plants should reduce the cost of lactic acid materially.

## USES FOR LACTIC ACID AND ITS DERIVATIVES

Lactic acid is a weak acid (Table 50), has a pleasant sour taste, and but little odor. It has good solvent properties, polymerizes readily, and most of its salts are highly soluble. Because of these properties, lactic acid has many uses both in food products and industrially.

TABLE 50. COMPARATIVE IONIZATION CONSTANTS FOR LACTIC ACID AND VARIOUS OTHER ACIDS

| Acid | $K \times 10^{-4}$ |
|---|---|
| Lactic acid | 1.38 |
| Formic acid | 2.14 |
| Acetic acid | 0.18 |
| Propionic acid | 0.13 |
| Tartaric acid | 8.00 |
| Citric acid | 9.60 |

Source: International Critical Tables, **6**, 258.

About half of the total lactic acid produced annually is of the edible grade. It is used as an acidulent in jams, jellies, confectionery, sherbets, soft drinks, extracts, etc. It is also used in brines for curing pickles and olives where it gives improved flavor and firmness in the finished product. Another application is for adjusting the pH of brewery worts to obtain proper saccharification and also to keep down undesirable bacterial fermentations. It is also added to cows' milk for infant feeding to improve digestibility.

The crude or technical-grade lactic acid has long been used in deliming hides. The high solubility of the calcium lactate which results from the action of the lactic acid on the lime in the hide and the mild effect of the lactic acid on the hide itself make it particularly satisfactory for this purpose. This deliming was originally done with a bran slurry which owed its effectiveness to lactic acid derived from the carbohydrates in the bran by the various lactic acid organisms present on the bran. The use of commercial lactic acid for this purpose produces a similar effect under better controlled conditions and more quickly. Lactic acid is also used in plumping leather but has little advantage over other agents used for this purpose.

The technical grade acid is also used in preparing the various esters, which are readily made by reacting the appropriate alcohol with lactic acid in the presence of suitable catalysts (sulfuric, phosphoric, or hydrochloric acid). The lower molecular weight esters, namely methyl, ethyl and propyl, are soluble in water, while the higher ones are water insoluble. They are all high boiling and useful as solvents and plasticizers in lacquers, varnishes, etc.

Lactic acid is used, to some extent, in textile and laundry work.[44] In finishing silk, rayon, and cotton, lactic acid produces better luster and smoothness of feel and affects breaking strength less than tartaric and other acids used. In dyeing wool with mordant and chrome colors, lactic acid is also superior because of its reducing action on bichromate.

In "souring" in the laundry, a process to avoid tarnish of linen on ironing due to incomplete removal of soap, etc., lactic acid is not harmful for the fabrics, even in considerable excess, and its salts are very soluble; as a result, it is particularly suited for this job.

The plastic-grade lactic acid has the following applications:

(1) It is used in the curing of certain phenolic resins.

(2) It is employed in the preparation of alkyd type resins[53]

formed by the reaction of polyhydric alcohols, such as glycerol, with dibasic organic acids, such as maleic, etc. Since lactic acid contains both hydroxyl and carboxyl groups, it can be reacted with the alcohol and increase the chain lengths and still have the same number of hydroxyl groups available for further reaction as the original alcohol had. In effect, it increases the chain lengths of the alcohol and the variety of polymers which can be obtained. These alkyd resins are widely used in paints, varnishes, and lacquers.

(3) In the methyl acrylate synthesis,[18,49] lactic acid can be esterified to yield the diester by reacting the $\alpha$-hydroxyl group with a suitable acid anhydride which can be pyrolyzed to the corresponding acrylic acid derivative. For example, methyl-$\alpha$-acetoxypropionate will yield the monomeric methyl acrylate on heating. The methyl acrylate will polymerize readily with heat or a suitable catalyst and has a wide range of uses in the preparation of lacquers, varnishes, impregnating agents for textile fabrics, paper, etc., as well as in plastics and molded compounds.

Since the first lactic acid process in America was developed for the production of calcium lactate for use in baking powder, it is interesting to note that this is still an important outlet for this acid and considerable quantities of calcium lactate are used in certain baking powders to stabilize them and control the rate of gas evolution. Calcium lactate is also used in certain pharmaceuticals as a source of calcium.

Sodium lactate is ordinarily sold as a 50% solution. It is strongly hygroscopic and during World War II was used extensively to replace glycerol in textile finishes, paper sizes, glue, tobacco, and for similar uses. In addition, it is a good freezing point depressant and gives a somewhat greater lowering of the freezing point in solutions than does an equal weight per cent solution of glycerol.[36] It is, however, somewhat unstable and inclined to be corrosive and, for this reason, attempts to commercialize its antifreeze properties have to date been unsuccessful. Further work on stabilizing sodium lactate[56] or possibly developing potassium or ammonium lactate for this purpose might yield a very suitable and cheap antifreeze. The various ethanolamine lactates have been considered as possible antifreeze solutions, but here again, the tendency to break down has prevented their commercialization.

During World War II, a pure grade of sodium lactate was developed and used for human transfusions where the victim had

lost large quantities of body fluids, such as in the case of severe burns.[38]

Copper lactate is used in electroplating by a new technique which makes it possible to obtain a range of finishes on metal by varying the conditions used. The finish is claimed to be permanent and to have many advantages over ordinary paint or enamel.

Its use is suggested in insecticides for chewing insects, such as a "Lactic Green"[14] as a possible replacement for Paris Green. Claims are also made for a fungicidal preparation for burlap and other fabrics prepared from a phenyl mercury salt and an excess of lactic acid.[52]

Calcium lactate has medical uses as a hemostatic and antispasmodic and as a source of calcium.

## GENERAL COMMENTS

The wide range of utility of lactic acid and its derivatives, both in the food and industrial fields, should greatly increase the demand for these versatile products. They are not without competitors, however, from other fields particularly synthetic chemical processes. Methyl acrylate, for instance, can be made from acetylene by the Reppe process or from ethylene chlorohydrin and sodium cyanide[43] at a price with which lactic acid cannot compete. However, lactic acid can be made largely from very low priced or even waste materials and, with the technology of manufacture of all grades quite well worked out, its manufacture on a large commercial scale, in well-designed plants should greatly reduce the production cost and extend the scope of its usefulness.

## BIBLIOGRAPHY

1. Allgeier, R. J., W. H. Peterson, and E. B. Fred, *Ind. Eng. Chem.*, **21**, 1039 (1929).
2. Anderson, A. A., and J. E. Greaves, *Ind. Eng. Chem.*, **34**, 1522 (1942).
3. Anon., Specifications for Lactic Acid, *U. S. Pharm. No. 12.*
4. Bernhauer, K., *Gärungschemisches Praktikum*, 2nd ed., Berlin, Julius Springer, 1939.
5. Bernhauer, K., J. Rauch, and J. N. Miksch, *Biochem. Z.*, **320**, 178 (1950).
6. Bonelli, A., and G. Gulinelli, The Industrial Preparation of

Lactic Acid from Sugar Beets, *Ind. chim. min. met.*, **5**, 121 (1918); *C. A.*, **13**, 133 (1919).
7. Borsook, H., H. M. Huffman and Yun-Pu Lin, *Biol. Chem.*, **102**, 449 (1933).
8. Braun, G., U. S. Patent 2,024,565 (1936).
9. Burton, L. V., *Food Inds.*, **9**, 571 (1937).
10. Byk, A., German Patent 278,487 (1912).
11. Carothers, W. H., G. L. Dorough, and J. H. Van Natta, *J. Am. Chem. Soc.*, **54**, 761 (1932).
12. Cordon, T. C., R. H. Treadway, M. D. Walsh, and M. F. Osborne, *Ind. Eng. Chem.*, **42**, 1833 (1950).
13. Cori, C. F., and G. T. Cori, *J. Biol. Chem.*, **81**, 389 (1929).
14. Dietz, Albert A., E. F. Degering, and H. H. Schopmeyer, *Proc. Indiana Acad. Sci.*, **51**, 76-7 (1941).
15. Ekelund, S. F. A., U. S. Patent 2,525,214 (1950).
16. Filachione, E. M., and C. H. Fisher, *Ind. Eng. Chem.*, **38**, 228 (1946).
17. Finn, R. K., H. O. Halvorsan, and E. L. Piret, *Ind. Eng. Chem.*, **42**, 1857 (1950).
18. Fisher, C. H., C. E. Rehberg, and L. T. Smith, *J. Am. Chem. Soc.*, **65**, 763 (1943).
19. Florsheim, N., German Patent 221,786 (1906).
20. Fred, E. B., and W. H. Peterson, *Ind. Eng. Chem.*, **13**, 211 (1921).
21. Fred, E. B., and W. H. Peterson, U. S. Patent 1,485,844 (1924).
22. Friedenberger, O., U. S. Patent 1,074,708 (1913).
23. Fries, K. W., U. S. Patent 2,474,046 (1949).
24. Garrett, J. F., *Ind. Eng. Chem.*, **22**, 1153 (1930).
25. Haag, I. L., and C. A. Vana, U. S. Patent 2,101,998 (1937).
26. Hillringhaus, F., and Heilmann, German Patent 171,183 (1905).
26a. Inskeep, G. C., G. G. Taylor, and W. C. Breitzke, *Ind. Eng. Chem.*, **44**, 1955 (1952).
27. Jenemann, J. A., U. S. Patent 1,906,068 (1933).
28. Kayser, E., *Ann. inst. Pasteur*, **8**, 736 (1894).
28a. Kitahara, K., and H. Ishida, *Bull. Research Inst. Food Sci., Kyoto Univ.*, No. 2, 27 (1949); *C. A.*, **46**, 4729 (1952).
29. Laszloffy, A. von, U. S. Patent 1,147,768 (1915).
30. Lawrie, J. W., U. S. Patent 1,594,843 (1926).
31. Lister, J., *Trans. Pathol. Soc., London*, **29**, 425 (1878).
32. Loder, D. S., U. S. Patent 2,365,945 (1942).
33. Needle, H. C., U. S. Patent 2,143,360 (1939).
34. Nelson, M. E., and C. H. Werkman, *J. Bact.*, **30**, 547 (1935).

35. Neuberg, C., and G. Gore, *Biochem. Z.*, **162**, 490 (1925).
36. Neuberg, C., and E. Reinfurth, *Ber.* **53**, 1783 (1920).
37. Nolte, A. J., and H. W. von Loeseke, *Fruit Prod. J.*, **19**, 204, 216, 220 (1940).
38. Olson, W. H., and H. Necheles, *Surg., Gynecol. Obstet.*, **84**, 283 (1947); *C. A.* **41**, 7526 (1947).
39. Orla-Jensen, S., N. C. Otte, and A. Snog-Kjaer, *Kgl. Danske Videnskab. Selskabs Skrifter, Naturvidenskab. math. Afdel.*, **6**, No. 5, 52 pp. (1936).
40. Owen, W. L., *Facts about Sugar*, **32**, 16 (1937); *C. A.* **31**, 7190 (1937).
41. Pan, S. C., W. H. Peterson, and M. J. Johnson, *Ind. Eng. Chem.*, **32**, 709 (1940).
42. Peckham, G. T., *Chem. Eng. News*, **22**, 440 (1944).
43. Pryor, C. C., *Petroleum Engr.* **13**, No. 8, 80 (1942).
44. Ranshaw, G. S., *Textile Colorist*, **64**, 356 (1942).
45. Scheele, K., Quoted by M. Bouillon LaGrange, *30 Germinal An. XII, Ann. chim. phys.*, **50**, 270 (1780).
46. Schopmeyer, H. H., and C. R. Arnold, U. S. Patent 2,350,370 (1944).
47. Smith, L. T., and H. V. Claborn, *Ind. Eng. Chem., News Ed.*, **17**, 370 (1939).
48. Smith, L. T., and H. V. Claborn, *Ind. Eng. Chem., News Ed.*, **17**, 641 (1939).
49. Smith, L. T., G. H. Fisher, W. P. Ratchford, and M. L. Fein, *Ind. Eng. Chem.*, **34**, 473 (1942).
50. Snell, E. E., *Wallerstein Labs. Commun.*, **11**, 81 (1948).
51. Snell, E. E., F. M. Strong, and W. H. Peterson, *Biochem. J.*, **31**, 1789 (1937).
52. Sowa, F. J., U. S. Patent 2,479,275 (1949).
53. Stearn, J. T., B. Makower, and P. H. Groggins, *Ind. Eng. Chem.*, **32**, 1335 (1940).
54. Stevens, K. R., and A. E. Western, "Potatoes as a Source of Lactic Acid by Fermentation," *Proc. Utah Acad. Sci.*, **19/20** 33 (1941-1943).
55. *U. S. Dept. Commerce, Special Report*, Nov. 10, 1950.
56. Vernon, W. H. J., F. Wormwell, H. G. Stroud, and H. C. K. Ison, British Patent 569,364 (1945).
57. Walsh, J. F., and H. C. Needle, U. S. Patent 2,143,358 (1939).
58. Ward, G. E., L. B. Lockwood, B. Tabenkin, and P. A. Wells, *Ind. Eng. Chem.*, **30**, 1233 (1938).
59. Wenker, H., U. S. Patent 2,334,524 (1944).
60. Werkman, C. H., *Ind. Eng. Chem., Anal. Ed.*, **2**, 302 (1930).

61. Werkman, C. H., *Iowa Agr. Exp. Sta. Ann. Rept., Pt. II,* 57 (1936).
62. Werkman, C. H., and A. A. Anderson, *J. Bact.,* **35**, 69 (1938).
63. Whittier, E. O., and L. A. Rogers, *Ind. Eng. Chem.,* **23**, 532 (1931).

CHAPTER 13

# THE CITRIC ACID FERMENTATION *

*Marvin J. Johnson*

Citric acid is a hydroxy tricarboxylic acid of wide occurrence in nature in many fruits. Its structural formula is:

$$\begin{array}{l} \qquad\quad CH_2\cdot COOH \\ \qquad\quad | \\ HO-C-COOH \\ \qquad\quad | \\ \qquad\quad CH_2\cdot COOH \end{array}$$

A quarter of a century ago, citric acid was obtained commercially only from citrus fruits. Today production has increased more than five-fold and approximately 90% of the product is obtained by mold fermentation. Production and import data for the prewar period have been summarized by Wells and Herrick.[67] American production of citric acid in 1947 was 30,000,000 lb, valued at $6,600,000.[55] Since 1950 great expansion of the production facilities for citric acid both in the United States and abroad has occurred and is continuing.[34a] Production during 1952 was probably at least 50,000,000 lb.

Mycological preparation of citric acid has been successful,

* The information referring to work of later date than early 1950 has been added by the editors.

because high yields of the acid can be obtained from a relatively cheap carbohydrate source, molasses. Citric acid, at a price of 27¢ per pound, occupies a position intermediate between such low-cost, large-scale fermentation products as ethyl and butyl alcohols and high-cost, low-yield products, such as vitamins and antibiotics. A commercially practicable synthesis of citric acid does not seem possible in the near future and, therefore, the fermentation citric acid industry is likely to continue its growth.

The development of the citric acid fermentation may be divided into three historical phases. The first phase, during which fermentations were carried on by various *Penicillium* species, with addition of a neutralizing agent, was begun by Wehmer[59] in 1893. The second phase, that of surface fermentations by means of *Aspergillus niger,* was inaugurated by the paper of Currie[15] in 1917. The third phase, that of submerged fermentation, began with the work of Perquin[37] in 1938 and of Karow[23] in 1942. The work done during the first phase can be dismissed as of historical interest only and will be referred to but briefly.

The German microbiologist, Carl Wehmer, discovered[59,60] in 1893 that certain molds would produce citric acid from sugar. He considered the isolated molds to belong to a new genus, *Citromyces,* although they have since been regarded as *Penicillia.*[43] In his publications,[61,62,63,64] Wehmer very apparently withheld exact information concerning the medium used, the conditions of incubation, and the yields obtained. With regard to sugar concentrations, he specifies "not too dilute" solutions or "10 to 20% sugar." Concerning other ingredients of the medium, he states only that calcium carbonate "may be added," or that it was used, and that ammonium nitrate, potassium phosphate, and magnesium sulfate (amounts unspecified) are added. The only yield figures given are that 11 kg of glucose yielded 6 kg of citric acid[59] and that yields "up to 50%" had been obtained.[64] The fermentation temperature specified is "ordinary temperature," although a statement is made that fermentation proceeds more rapidly at 15° to 20°C than at lower temperatures. The fermentation time specified is 8 to 14 days[61] although Wehmer later[64] stated that 4 to 6 weeks were required. The latter figure is more in agreement with the results of other workers with similar cultures.

The reason for Wehmer's vagueness with regard to experimental details was undoubtedly his intention to make industrial use of the

fermentation. An attempt was made to utilize Wehmer's process commercially at a factory in Thann, Alsace, beginning in 1893. It was not successful and was abandoned in 1903.[37] The difficulties encountered were later summarized by Wehmer.[64] Illustrative of the fact that some of the difficulties involved in commercial fermentations have not altered since 1893, some of Wehmer's difficulties may be listed: (1) difficulty of selection of proper organisms, (2) degeneration of the organism, (3) contamination, (4) inordinately long fermentation time, (5) high plant construction costs, (6) insufficient spread between raw material costs and price of finished product. It is interesting to note that a significant omission in this list is the difficulty of maintaining an optimal medium at all times, a difficulty which certainly exists in all modern citric fermentations, but which was probably overlooked by Wehmer. Wehmer writes that, up to 1910, no successful citric-fermentation process had been evolved in Germany. This statement is confirmed by a consular report of the same year.[54]

Other investigators, not so chary of disclosing experimental procedure, followed Wehmer. Mazé and Perrier[30,31] isolated and used *Penicillium*-like mold strains which they named as members of the genus *Citromyces* for no apparent reason apart from the fact that they produced citric acid. They used glucose concentrations of the order of 10% and organic nitrogen sources. Their best medium was a bean extract. Calcium carbonate addition was found to be essential to high yields. The incubation temperature used was 16° to 22°C. The highest yield reported (after 57 days incubation) was 4.47 g citric acid from 11.63 g added glucose in 100 ml medium. They showed, however, by serial analyses that incremental yields of more than 50% of sugar used were obtained after mycelial growth was essentially complete. Of interest is the fact that they obtained considerable amounts of citric acid from glycerol. They came to the conclusion that citric acid was not a direct product of sugar metabolism, but was liberated into the medium by autolyzing mycelium.

Buchner and Wüstenfeld[9] used cultures obtained from both Wehmer and Mazé. They obtained yields up to 69% of sugar used, after 66 days of incubation. Herzog and Polotzky,[22] using "*Citromyces*" strains isolated by themselves, obtained yields up to 28% on 5% glucose solutions in 8 weeks. Their yields from glycerol were

just as good as those from glucose. They also obtained citric acid from a variety of hexoses, pentoses, and disaccharides.

Neither Wehmer nor the workers who followed him discovered that *Aspergillus* species form citric acid. These molds were believed to yield primarily oxalic acid. It is difficult to guess what the reason may have been for this failure to find citric acid as a product of sugar oxidation by *Aspergilli.* One possibility is that the media used (including the popular Raulin's medium) contained zinc, iron, manganese, and phosphorus in sufficient amounts to prevent citric acid accumulation in *Aspergillus* cultures. As will be brought out later, only in media deficient in some of these elements does citric acid accumulate.

The second phase of the history of citric acid fermentation began with the publication in 1917 of Currie's excellent paper on citric acid production by *Aspergillus niger.*[15]

Currie's investigation was the result of the observation of Thom and Currie,[52] during a study of oxalic acid production by *Aspergilli,* that the observed titrable acidity sometimes greatly exceeded the oxalate produced. The acid present was identified as citric, and Currie's work followed. Currie's high yields of citric acid, it appears in the light of present knowledge, were probably due to his recognition that minor constituents of the medium were important from the standpoint of citric acid yield. He mistakenly believed that no mineral elements other than potassium, phosphorus, magnesium, and sulfur were necessary for mold growth, but his conviction that the usual mold media were too complex undoubtedly led him to use a medium sufficiently simple to make possible systematic variation of its components.

Currie's discovery was, essentially, that many strains of *Aspergillus niger,* when grown at low pH values in surface culture on concentrated (up to 15%) sucrose solutions, in inorganic media containing optimal concentrations of certain nutrients, gave yields of citric acid up to 55% of the added sucrose. His recommended medium contained the following, in grams per liter: sucrose, 125 to 150; $NH_4NO_3$, 2 to 2.5; $KH_2PO_4$, 0.75 to 1.0; $MgSO_4{\cdot}7H_2O$, 0.2 to 0.25; and HCl to pH 3.4. His fermentations were carried out in 200-ml Erlenmeyer flasks, containing 50 ml of medium, or in shallow pans. Incubation was at 28°C, generally for 8 days. Currie apparently experienced the lack of reproducibility of results that has been characteristic of most, if not all, studies of the citric acid

fermentations. He stated that "the variability of the fermentations under what appears to be identical conditions is a difficulty that has not been entirely overcome. . . . All of the conditions that may influence the course of the fermentation are not within the control of the experimenter."

With the work of Currie, the modern period in the investigation of citric acid formation by molds may be considered to have begun. Some of the results obtained by the host of workers who followed him will be discussed in the following sections.

## FACTORS AFFECTING CITRIC ACID PRODUCTION BY *A. NIGER* IN THE LABORATORY

A large number of workers have contributed to our knowledge of the citric fermentation. The laboratories of Bernhauer,[2,3,4] Butkevich,[10,11] and Chrzaszcz[12] have been especially active.

On some points, there is general agreement among laboratories. The *Aspergilli* have been found to be the best citric acid producers. Special strains of *A. niger* are generally used, but others, such as *A. awamori*[32] and *A. wentii*,[24,56] have been also employed. The cultures are best stored as dry spores, without periodic transfer.[3] The mold may be grown on a growth medium, then made to produce citric acid on a replacement medium,[10,24] or citric acid may be produced in the original medium.[48,68] The first procedure appears best for maximum yields for the purpose of mechanism studies,[10] but the second is generally found superior for highest practical yields, that is, grams of citric acid produced per gram of total sugar used, including sugar used for growth. The depth of medium used for the surface-growth procedure is generally between 1 and 2.5 cm, although depths of 5 cm[17] and 8 cm[7] have also been employed.

### Fermentation Temperature

For *A. niger* fermentations, an incubation temperature of 28° to 30°C has been generally found optimal. Bernhauer,[2,4] however, used higher temperatures, 32° to 35°C. Kovats[25] found that best results were secured in sucrose fermentations when the temperature was held at 28°C during the first 3 days, then reduced to 20°C. With beet-molasses media, however, he found[27] that the optimum temperature depended on the acidity of the medium. For sub-

merged-culture fermentations, Szücs[50] and Shu and Johnson[48] used 25°C, and Karow and Waksman[24] used 28°C with *A. wentii.* The optimal incubation temperature is undoubtedly a function of the mold strain and the medium used and should be determined for each set of conditions.

## Sugar Concentration

Sugar concentrations of more than 20% have been used, but there is general agreement that concentrations of 10 to 20% are optimal. Shu and Johnson,[48] working with submerged fermentations, found that concentrations of 26% were not too high since the time required for fermentation was proportional to the sugar concentration. There is some evidence that the use of high sugar concentrations is more successful when the citric acid is partially neutralized by the addition of calcium carbonate or other neutralizing agents.

## Organism Used

There is general agreement that only selected strains of *A. niger* yield large amounts of citric acid. All workers have found that among high-yielding strains, there are differences with regard to the optimal conditions for the fermentation. As a rule, the same strain is not found best in different laboratories, probably because of differences in fermentation conditions. In a field where each worker uses his favorite culture, it is very difficult to compare the work of various laboratories or to draw general conclusions based on the results of more than one investigator. The fact that workers do not agree on the merits of a given strain is evidence in itself that a number of unknown variables are involved in the experimental conditions used in various laboratories. Even the use of distilled water from more than one still has been shown, on more than one occasion, to cause variability of results. Different lots of sugar also often give different results. It is probable that the reason best results are most often obtained with sucrose is the fact that this sugar is most readily available in high purity. Studies of the relative yields of citric acid from various sugars are of no value unless it can be shown that impurities in the sugars used have no effect.

It has been found repeatedly that a culture will vary with time in its ability to give high yields of citric acid. The author, however,

is inclined to consider at least part of the variability attributed to changes in the culture as due to unrecognized changes in the composition of the medium or in other conditions. It is well known that mold cultures transferred at regular intervals by spore transfer are likely to change their characteristics. It is equally well known that very little, if any, change takes place in a mold culture stored in the form of dry spores.

### Yields Obtainable

The citric acid yields reported by various workers, of course, vary greatly. When citric acid is produced by preformed mold mycelium, the actual yield can usually not be determined, since the sugar used in growing the mycelium is often not determined. When citric acid is produced in the growth medium, the yield has been, in most of the studies mentioned, between 60 and 80 g of anhydrous citric acid per 100 g of added sugar. When crude sugar sources, such as beet molasses, are used, the yields generally range from 50 to 70%. There is no standard manner of expressing yield. The basis used may be citric acid monohydrate (the commercial form of the acid), or anhydrous citric acid. It may be calculated on sugar used or sugar added. The sugar used for development of mycelium may or may not be included. Citric acid may be determined colorimetrically, by isolation of calcium citrate, or by simple titration, with or without correction for the oxalic acid often produced in appreciable amounts. These factors must be considered in evaluation of yield figures given in the literature.

### Composition of the Medium

Apart from the selection of a suitable culture, the use of a medium of the proper composition is certainly the most important condition for realization of high citric acid yields. A medium giving optimal growth of the fungus does not give good citric acid production, since carbon converted to mycelium is unavailable for citric acid formation. It may be safely assumed that since carbon dioxide is the normal end product of carbohydrate utilization, conditions under which citric acid accumulates are abnormal and may be considered to be the result of nutritive deficiencies in the medium. The media used by most workers are similar in that the great majority of them contain chiefly ammonium nitrate, monopotassium phosphate, and magnesium sulfate. Hydrochloric acid

is commonly used to lower the pH. Metal ions (usually iron, zinc, and manganese) are sometimes added. There is, however, no agreement with regard to the optimal amounts of some of the constituents. The amount of ammonium nitrate used does not vary greatly, 2 to 3 g per l, or an equivalent amount of some other nitrogen-containing salt, being used. This limits mycelial growth to 10 to 20 g per l. Less growth may be obtained if some factor other than nitrogen is the limiting nutrient, as is often the case. The amount of monopotassium phosphate used is either high (1 to 3 g per l) or low (0.3 g per l). The amount of magnesium sulfate used shows great variation. Addition of metallic ions is found to be harmful, helpful, or without effect, depending on the investigator and the experimental conditions.

Although the published data on the effect of the composition of the medium are complex and contradictory, it is possible to form some conclusions which appear to be consistent with most of the data. Even if these conclusions serve only as an aid in orienting the mass of available data, they are of value. It is generally agreed that a mold culture with an abundance of nutrients will not accumulate citric acid, but will oxidize sugar completely to carbon dioxide and water. Therefore, it is probable that citric acid accumulation occurs as the result of an enzymic deficiency caused by the use of a medium deficient in one or more essential nutritive elements. The literature indicates that several types of deficiencies lead to citric acid accumulation. Among the nutrients apparently of importance in this respect are phosphate, manganese, iron, zinc, and probably copper. There are several reasons for the unsatisfactory state of our present knowledge. First, very few investigators have given attention to the trace metals present as impurities in their media. Second, those investigators who have systematically varied constituents of the medium have almost always used as a reference medium (the constituents of which were varied one at a time) one which gave optimal citric acid yield and thus was already deficient in one or more essential components. The complex deficiencies resulting from this procedure are difficult to interpret. Third, a large number of different strains of *A. niger* have been used. Since it is certain that the nutrient requirements vary with the strain used, comparison of the work of different investigators is difficult.

In spite of these difficulties, some degree of consistency can be

found in the seemingly contradictory reports in the literature if the assumption is made that citric acid accumulation occurs when the medium is deficient in one or, preferably, more than one, of the following: phosphate, manganese, iron, zinc, and probably copper.

In a part of the work of Shu and Johnson[47] on submerged citric acid formation by *A. niger,* a medium not sufficiently deficient to bring about accumulation of significant amounts of citric acid was used. This medium was deficient in manganese, however. The constituents of this medium were varied one at a time, others being present in excess. It was found that citric acid accumulated in fairly large amounts when phosphate, iron, or zinc were made limiting. In each case, the concentration of the limiting nutrient which gave optimal acid production was one at which mycelial growth was reduced somewhat, but not greatly. In the complete medium, the limiting nutrient for mycelium production was nitrogen. Further limitation in nitrogen, or production of a magnesium deficiency, did not result in citric acid accumulation. It had previously been found[48,46] that citric acid would not accumulate in any of the media tried unless manganese was deficient. Best yields were obtained on a medium deficient in iron, zinc, and manganese. Combinations of phosphate deficiency with these deficiencies were not tried. Copper was not varied and may have been present in slightly deficient amounts.[45]

Tomlinson, Campbell, and Trussel,[53] who used the same strain of *A. niger* as Shu and Johnson, but used the surface-culture method, report that "the quantities of iron, copper, and manganese found most suitable for the production of high yields of citric acid were lower than the quantity of those elements required for good growth." They also found that zinc was necessary, but that excessive amounts decreased yields.

Perquin,[37] Szücs,[50] and Karow and Waksman,[24] working with replacement media in submerged culture, all stress the importance of low phosphate levels for high citric acid yields. The first two workers presented no data on the metal content of their media: Karow and Waksman added zinc and manganese. Citric acid accumulation apparently occurred because of phosphate deficiency or a combination of phosphate and metal deficiencies. More recently Szücs[51] has found the addition of skim-milk solids to be advantageous. Since many investigators have found that organic growth factors are not required by *A. niger* for either growth or

citric acid production, it might be assumed that the supplement either provides an element present in suboptimal quantity, or removes (by combination with protein) an element present in adequate amount, thus producing a desirable deficiency.

A number of investigators, using the surface-culture method, have also apparently used phosphate deficiency to bring about citric acid accumulation. Among them are Butkevich and Timofeeva,[11] Wells, Moyer, and May,[68] and Nakazawa, Takeda, and Nakano.[32] Very high yields were obtained by Butkevich and Gaevskaya[10] by growing the mycelium on a medium very low in phosphate and using a replacement medium containing no added phosphate.

It is unquestionably true that mold strains vary in their quantitative requirements for essential elements. In addition, the degree of deficiency of one metal which is optimal for citric acid production depends on the degree to which other metals are deficient. Thus, Perlman, Dorrell, and Johnson[35] found with *A. niger* strain 62, on a medium apparently deficient in iron, zinc, and manganese, that partial removal of the iron deficiency or of the manganese deficiency would increase yields, but that when optimal amounts of iron were added, addition of manganese decreased yields. Addition of zinc always decreased yields. Shu and Johnson[47] found that the degree of iron deficiency giving optimal yields depended on the degree of zinc deficiency present.

Most workers who have investigated the effect of additions of metals have not adopted the technique of investigating each metal when the others were present in excess. Metal ions have been added to a basal medium containing unknown amounts of metal ions as impurities, or, if it is found that addition of one metal at a certain level is optimal, addition of further metals is tried with a medium containing optimal amounts of the first metal. Since the trace-metal content of the sugars and salts used in the media of most workers was not determined, it follows, in the case of investigators who used relatively high phosphate concentrations and who, therefore, brought about citric acid accumulation by means of uncontrolled metal deficiencies, that effects observed when the concentrations of salts and sugars were varied may have been due to accompanying variations in the trace-metal content of the medium. Thus, the observation of Doelger and Prescott[16] that increasing the magnesium sulfate concentration above 0.3 g per l gave sporulation and oxalic acid production may have been related to the manganese

content of the salt used. Likewise, their finding that substitution of glucose for a part of the sucrose in the medium gave reduced yields may be explicable on the basis of the metal content of the sugars used.

In general, the results in the literature indicate that most workers have used media deficient in either phosphate or metals. It is probable that Currie's original success in obtaining citric acid production by *Aspergilli* was due to his belief[15] that the trace elements usually added to growth media were not necessary. Currie used a medium apparently containing ample phosphate. He found that the addition of 1 or 2 mg of iron per l increased his yields slightly, but that there was no decrease in yield even at 20 mg iron. Since no zinc or manganese was added to any of his media, it appears probable that deficiencies in these metals were responsible for his success. Butkevich and Timofeeva[11] used a medium which contained 11 mg zinc and 10 mg iron per l and thus was not deficient in these elements. Manganese was neither added nor determined. They were able to obtain good yields by making the medium deficient in phosphate, or in phosphate and nitrogen. Most other workers have been unable to increase yields by nitrogen deficiency unless the original nitrogen content was so high that an inordinate amount of sugar was utilized in the synthesis of mycelium. They were also able to increase yields by production of a sulfur deficiency. Chrzaszcz and Peyros[12] found iron to increase yields, but zinc to reduce yields. Apparently the iron content of their unsupplemented medium was so low that the optimal iron and zinc deficiency was obtained only with added iron. They found a great difference between strains with regard to the effect of iron. Kovats,[25] using a medium containing an excess of phosphate and tap water, found that iron decreased his yields, but that zinc had no effect. Quilico and Di Capua[40] found that 0.65 mg iron per l reduced the yield to one-tenth of that obtained without iron. Giordani[21] also found that iron reduced yields. In both of these investigations, ample phosphate was present. Porges,[38] however, found that addition of iron was beneficial. For submerged fermentation, Karow and Waksman[24] added zinc and manganese to their low phosphate-medium, but did not investigate the effect of iron. Schweiger and Snell[44,49] used a low-phosphate medium for submerged fermentation and stated that the iron content must not be more than 1 mg per l. Even with this amount of iron, zinc must

be added to "counteract the effect of iron on cell morphology."

Recently, Moyer[31a] described a novel approach to the problem. He included 1 to 3% (v/v) methanol in his media and reported good citric acid yields in submerged culture studies, with increased tolerance of *A. niger* to zinc, iron, and manganese. Methanol does not appear to be metabolized and the best concentration is slightly toxic. Both molasses and glucose media were studied.

It seems probable that the reasons for the divergent results obtained by different investigators are due in part to differences in metal requirements among strains and to variations in the known and unknown constituents of media used. For example, if iron were present (as an impurity) in a medium at a level too high to give optimal citric acid accumulation, addition of iron would be harmful. If only very small amounts of iron were present, addition of iron would be beneficial. If relatively large amounts of both iron and zinc were present, and citric acid accumulation were brought about by phosphate deficiency, addition of iron would have no effect. If citric acid accumulation were brought about by a multiple deficiency, the behavior on addition of one nutrient would be difficult to interpret, especially if the nutrient varied (for example, ammonium nitrate) were contaminated with trace metals.

## COMMERCIAL PRODUCTION OF CITRIC ACID

### Use of Crude Carbohydrates as Carbon Sources

It is apparent from the preceding discussion of the necessity for a deficient medium for citric acid production that the use of crude carbohydrates, containing large amounts of various nutrients, would present difficulties. The crude carbohydrate sources which have received attention are beet molasses, cane blackstrap molasses, and cane invert molasses. Most success has been obtained with beet molasses.

It is often difficult to determine from the published work on molasses media the type of deficiency to which citric acid accumulation is due. In many cases, however, the type of deficiency achieved is obvious. For example, Quilico and Di Capua[41] used beet molasses without treatment to remove metals. They found that optimum yields (50 to 55% of available sugar) were obtained when 0.01 to 0.02% monopotassium phosphate was added to the medium. If the monopotassium phosphate content was raised to 0.1%, yields were

reduced several fold. If the total phosphorus content of the molasses used is regarded as being available to the mold, the total effective monopotassium phosphate content was approximately 0.03%, a value in good agreement with the phosphate levels found optimal by investigators using purified sugars in phosphate-deficient media. Perlman,[33] working with a ferrocyanide-treated beet molasses, found that yields were reduced manyfold when phosphate (0.29 g $H_3PO_4$ per l) was added to the medium. The inorganic phosphorus content of the molasses he used was 0.0295 g per l of medium, but the total phosphorus was much higher, 0.44 g per l of medium. The phosphate content of the medium used in the German citric acid plant at Ladenburg,[7] which used beet molasses, was rigidly controlled, and the phosphate concentration was realized to be of great importance. The amount of phosphoric acid used depended on the phosphorus content of the molasses being used and the medium apparently was held at a level equivalent to approximately 0.3 g monopotassium phosphate per l.[8] Ferrocyanide was also used. Kovats,[26] using untreated beet molasses, found that addition of phosphate lowered the yields.

It is thus evident that beet-molasses fermentations may sometimes be successfully carried out by making the medium phosphate deficient. In most cases, however, apparently metal deficiencies, or combined metal and phosphate deficiencies have been used. Metal deficiency, in molasses fermentations, may be brought about by treatment with ferro- or ferricyanide, by cation-exchange processes, or possibly by adsorbents. The most usual method of reducing the metal content of beet molasses before fermentation is by treatment with potassium ferrocyanide.[4,7,20] The usual procedure is to determine for each lot of molasses the optimum amount of ferro- or ferricyanide by determination of the yield of citric acid as a function of treatment level. The pH at which the treatment is carried out is also important. Bernhauer, Rauch, and Gross[4] found pH 5.8 to be optimal; Gerhardt, Dorrell, and Baldwin[20] obtained best results between pH 6 and pH 8; at the Ladenburg works[7] a pH of 7.5 was used; and in a German plant at Ingelheim,[8] a pH of 6 to 6.5 was employed.

It is likely that the chief effect of ferrocyanide is to reduce the iron content of the molasses to a point where citric acid accumulation through iron deficiency becomes possible. Bernhauer *et al.*[4] found that addition of iron to the treated medium in an amount

sufficient to restore the original iron content resulted in very poor citric acid yields. It is possible, however, that ferrocyanide also reduces the content of other metals to a level corresponding to a deficiency optimal for citric acid production.

Cationic exchange has also been used to reduce the metal content of beet and cane molasses for citric fermentation. Perlman, Kita, and Peterson[36] were able to obtain yields up to 42% on invert molasses after treatment with cationic-exchange substances. Their medium was not deficient in phosphate. Karow and Waksman,[24] using a phosphate-deficient medium and submerged fermentation, found ion exchange increased citric acid yields on invert molasses. Woodward, Snell, and Nicholls[70] conditioned invert molasses for citric fermentation through removal of iron by ion-exchange resins. Schweiger and Snell[44,49] describe a citric acid fermentation process (submerged fermentation), using a low phosphate medium, in which ion exchange is used to remove iron from molasses.

Adsorbents have also been used to purify molasses for fermentation, but have not been as successful as ion exchange and have the disadvantage of high cost. The most successful methods for fermentation of beet molasses have been ferrocyanide treatment, often combined with phosphate deficiency. Oxalic acid formation is more likely to prove a problem than in the case of synthetic media, probably because of defective achievement of optimal deficiency conditions. For cane invert molasses, ferrocyanide treatment has been successful,[36] but no study has been made of the effect of phosphate deficiency in combination with ferrocyanide treatment.

## Commercial Surface Fermentation

Until very recently, all citric acid production was by means of the surface-culture method. It appears probable that submerged culture will largely supplant it in the future. The chief carbohydrate source is beet molasses, although some cane molasses has also been used. Details of the processes used industrially are not divulged, and patent information is of doubtful value, because most of the patents have been assigned to firms not manufacturing citric acid and because of the well-known practice of disclosing as little information as possible in a patent. The unwary reader of the

patent literature might conclude that there were any number of commercially practicable procedures for obtaining high yields of citric acid from crude carbohydrate materials. The fact that until very recently only one company in the United States produced citric acid by fermentation, however, indicates that efficient commercial processes are not plentiful.

The procedures used by two commercial citric acid plants in Germany have been described. The processes certainly do not appear optimal, particularly from a microbiological standpoint, but a description of them is of unique value because it constitutes the only published information concerning actual commercial citric acid fermentation processes.

The process in use at the Benckiser works at Ladenburg has been most completely described.[6,7,8] The plant began operating only in January 1945 and, because of wartime conditions, was never operated at its full capacity of 6,000 to 10,000 kg of calcium citrate a day. It produced crude calcium citrate, which was to be shipped to Ludwigshafen for conversion to citric acid. The plant for conversion, however, was not finished before the German collapse. The Ladenburg process was apparently based, to some extent, on that of the Montan und Industrialwerke, at Kasnejov near Pilsen. The strain of *A. niger* used was one improved by selection from a culture obtained from Bernhauer. Spores grown on molasses agar were used as inoculum. Beet molasses, containing 48 to 50% sugar, preferably from plants producing raw sugar, was used. Each lot of molasses was analyzed for iron, phosphorus, and nitrogen. A series of laboratory fermentations was run on each lot of molasses to determine the optimum amount of phosphoric acid to be added. Laboratory tests also determined the amount of ferrocyanide to be used. There is some disagreement among the various accounts[6,7,8] regarding the procedure used in preparing the molasses for fermentation. The molasses was diluted to 30% sugar, adjusted to neutrality with sulfuric acid, treated with ferrocyanide and phosphoric acid, heated at 100°C for sterilization, and diluted to 15% sugar for fermentation. The amount of phosphoric acid added was sufficient to bring the $P_2O_5$ content of the molasses to at least 0.02%. The treated molasses was then run into the fermentation pans. There were 20 fermentation chambers, each containing 80 aluminum trays of $2 \times 2.5$ m size and 15 cm deep. They were filled to a depth of 8 cm with the diluted molasses.

inoculated by means of spores blown in with the air supply, and incubated 9 to 11 days at 30°C. The mold mats were removed by hand and extracted, 15% of the total yield being obtained from the washings. The fermentation liquor was heated, treated with calcium oxide to pH 8.5, and the precipitated crude calcium citrate filtered off.

The air supply for the fermentor chambers was "sterilized" by passage through a cotton filter, 2 in. thick impregnated with salicylic acid, then passed through a water spray and heaters to bring it to 40% humidity at 30°C. Air was supplied to the fermentation chambers at the rate of one volume of air per volume of medium every 4.3 minutes.

The fermentation chambers were sterilized by washing with 1% caustic soda, then with water, then with 6% formaldehyde. Finally, sulfur dioxide was blown into the chambers with the air stream.

The weakest feature of the process, if available accounts are reliable, appears to be the inadequate provisions for sterilization and asepsis. After cooking, the molasses medium was cooled by dilution with well water. The air supply was certainly not sterile and apparently it was usual for operators to enter the fermentation chambers during the progress of the fermentation. It is claimed, however, that very little trouble from contamination was experienced. It might be expected that if an organism capable of competing with *A. niger* under the environmental conditions used once became established, it would be extremely difficult to eliminate.

The yield claimed was 70% of the added sugar as recovered yield of citric acid, presumably monohydrate. This yield, if reliable, certainly appears to reflect excellent fermentation and recovery procedures.

Another German plant on which data are available[8] is that of C. H. Boeringer Sohn, at Ingelhein on the Rhine. The only indication of the capacity of the plant is that there were twelve fermentation chambers, some containing 100 trays, some 72, and some 54. The trays were 2 × 2.5 m in size and 12 cm deep. They were of steel, brush-painted with a rubber-containing lacquer. Maintenance costs were said to be heavy. The plant began fermentative production of citric acid in 1938-39.

The procedure used was, in general, similar to that used at Ladenburg. The culture of *A. niger* used was said to be a special isolate. No ferrocyanide was used if the ferric oxide content of the

molasses was below 0.03%. Contamination was admitted to be a major problem. In this plant, the mold mat was not handled separately, but was broken up and run out of the pans with the fermentation liquor. Calcium citrate was precipitated at neutrality for 3 to 4 hours at 80° to 90°C, filtered off in a filter press, and converted to citric acid by the addition of 1% excess of sulfuric acid. Activated carbon and ferrocyanide (for removal of iron from the product) were added, the calcium sulfate removed in a filter press, and the liquor concentrated in vacuum pans at 40° to 45°C, lanolin being used as an antifoam agent. After concentration to about 800 g per l, the liquor was placed in agitated crystallizers cooled with well water. The crystalline product was recovered in centrifugal separators, and recrystallized once. Mother liquor from the first crystallization was again evaporated and crystallized. The fermentation yields were said to be 60%, of which 10% was lost in recovery.

In these plants, the medium used was evidently one deficient in phosphate and iron. Data on the manganese content of the medium and on the effect of ferrocyanide treatment on the manganese content would be of interest. From information available in the literature, it is difficult to conclude what types of deficiencies are likely to be feasible in beet-molasses fermentations. Both simple-phosphate deficiency[38] and deficiency in both phosphate and metals have been used. Lack of data on the phosphate content of the molasses samples used makes it difficult to determine whether metal deficiencies alone have been successfully used, but in all probability, such a procedure is possible.

No successful surface fermentations have been reported in which cane molasses, either blackstrap or invert, has been used as the carbohydrate source. Whether the explanation lies in the metal or in the phosphorus content of the molasses is difficult to determine. Since cation-exchange procedures have been shown to improve the yields from such molasses,[36,49,70] it is certain that at least a part of the difficulty lies in its metal content.

## Commercial Submerged Fermentation

In many aerobic fermentations, such as the production of gluconic acid, penicillin, and riboflavin, the advantages of submerged culture over surface culture are so obvious and so well known that they need not be discussed in detail. Among them are

lower labor cost, higher yields, shorter time cycle, simpler operation, and easier maintenance of asepsis under industrial conditions. These advantages can be realized by submerged operation of the citric fermentation only with a process by means of which crude carbohydrate sources, in high concentrations, can be rapidly converted to citric acid in equipment that is not unreasonably expensive because of corrosion, contamination, and metal-toxicity problems. These objectives have not been achieved in any published study. A great deal of progress has been made, however, and a new citric acid plant, employing the submerged-culture process, went into production during 1951.[1] It is possible that better procedures will continue to be developed.

The first submerged culture giving good yields was described by Szücs.[50] He used pure sucrose. The mycelium was grown on a medium apparently phosphate deficient (0.3 g monopotassium phosphate per l), and the mycelium transferred to a new medium containing no phosphate, on which the citric acid was produced. The growth phase required 3 days, and the citric acid forming phase 4 days. The cultures were incubated at 25°C with agitation and under oxygenation with pure oxygen. In a later patent,[51] Szücs described a modified procedure. Air, rather than oxygen, was used for aeration and a single medium was employed. This was phosphate deficient (0.15 g monopotassium phosphate per l) and contained 160 g pure sucrose per l. Dried skim milk was added to the medium at a level of 0.5 g per l. A yield of 92% is claimed, the fermentation requiring 9 days. The great improvement in the fermentation brought about by the skim milk may perhaps be attributed to removal of trace metals.

The long fermentation time and the need for crystalline sugars make the Szücs procedure of doubtful commercial importance. The procedure of Shu and Johnson[46,47,48] also requires crystalline sugar and involves a long fermentation time, although the use of larger fermentors in place of shake flasks might reduce the time considerably. Shu and Johnson used iron, zinc, and manganese deficiency in place of phosphate deficiency. Their medium contained ample phosphate, but special purification procedures were necessary to reduce the concentration of metals, especially manganese. They, of course, used a strain of *A. niger* different from that used by Szücs.

Buelow and Johnson[9a] adapted the procedures of Shu and

Johnson to 100-l fermentations in 50-gal glass-lined tanks to determine the aeration requirements under these conditions. Yields of 70 to 80 g of anhydrous citric acid per 100 g added sucrose were obtained from 15% sucrose solutions in 160 to 240 hours. The fermentation time was decreased when the effective aeration rate was increased.

Martin and Waters[28a] employed the same organism as the Wisconsin workers in studying the submerged-culture fermentation of crude beet molasses on the laboratory scale. Mashes were prepared by diluting the molasses to 12% sugar, adjusting the pH to 6.0, sterilizing, and adding potassium ferrocyanide and monopotassium phosphate supplement. The levels of ferrocyanide and phosphate required varied with the molasses and were determined approximately by shake-flask experiments. Fermentations were conducted in tower-type fermentors with vigorous aeration for 24 hours, then vigorous oxygenation with pure oxygen to the end of the fermentation period. The highest yield was 72 g of anhydrous citric acid from 100 g of available sugar in 70 hours. These submerged citric acid fermentations were the most rapid that have been reported.

*Aspergillus wentii* is used in the submerged fermentation process of Karow and Waksman.[24,56,57] In this procedure, as in the original procedure of Szücs, a growth medium and a fermentation medium are employed—both phosphate-deficient—and pure oxygen is used for aeration. Pure sugars are used, but some success was obtained with treated molasses. The process does not appear practicable because of the long fermentation time (8 to 10 days) and the need for crystalline sugar. The degree of treatment necessary for molasses appears commercially unsuitable.

The Miles Laboratories[44,49,70] appear, from the results claimed in their patents, to have approached a commercially feasible submerged process. A plant to use this process has been put into operation by this company.[1] From the patents, it is seen that they use a medium deficient in both phosphate and iron. Manganese may also be deficient. They are able to use invert molasses after pretreating it with a cation exchanger. They also claim[44] that addition of morpholine is beneficial. However, the fermentation period, 9 days, specified in one patent, is long. Undoubtedly their plant fermentation period is shorter.

Moyer,[31a] using methanol as a supplement in commercial

glucose or molasses media, appears to have increased the tolerance of *A. niger* to zinc, iron, and manganese. Submerged-culture studies were made in flasks and in stainless-steel tanks. A citric acid yield of 64.4% on the glucose consumed was reported for an 8-day fermentation, using a medium containing per liter: 114g anhydrous glucose, 0.1g $MgSO_4 \cdot 7H_2O$; 0.3g $KH_2PO_4$; 0.32g $Mg(NO_3)_2 \cdot 6H_2O$; 1.8g $NH_4NO_3$; 0.044g $ZnSO_4 \cdot 7H_2O$; 0.01g $MnSO_4 \cdot 4H_2O$; and 0.25ml corn-steep liquor. The initial pH was 4.1. To this medium, 2% methanol (v/v) and 2% germinated inoculum were added at the beginning of fermentation.

Sufficient work has been done on submerged-culture processes to make it appear reasonable that commercially feasible processes will be developed. The use of an organism, perhaps an induced mutant, with metal requirements so high that deficiencies are readily produced, or of an organism with a defective enzyme system which would accumulate citric acid even in a complete medium, is certainly desirable. Fermentation times can probably be reduced by the use of optimal aeration and agitation equipment. The use of a medium on which relatively heavy growth is produced would shorten the fermentation time, but reduce the yield, because of the large amount of sugar carbon converted to mycelium. The citric acid fermentation, since it is less subject to contamination than most commercial aerobic fermentations, should be suitable for continuous operation. Such a procedure should decrease fermentation time very considerably.

## MECHANISM OF CITRIC ACID FORMATION BY MOLDS

Of prime importance in a discussion of the mechanism of citric acid production is the percentage of sugar carbon convertible to citric acid. Highest yields should be expected on a replacement medium where no sugar carbon would be utilized for production of mycelium. However, in most experiments of this kind, the possible contribution of mycelial carbohydrate to citric acid production has not been controlled. In experiments in which the acid is produced on the growth medium, however, approximate correction may be made for the carbon of the mycelium. Wells, Moyer, and May[68] have shown in this manner that at least five-sixths of the carbon of glucose appears as citric acid. The same result is obtained by calculation from the data of Shu and Johnson.[48] Since a mechanism yielding five out of six carbons as citric acid appears

somewhat improbable, the most likely mechanisms are those yielding 1 mole of citric acid from 1 mole of glucose.

Suggested mechanisms of only historical interest will not be discussed. In mechanisms yielding 1 mole of citric acid from 1 mole of glucose, it is postulated that a four-carbon compound condenses with a two-carbon compound, usually acetic acid. Thus, Raistrick and Clark[42] considered acetic acid to condense with oxalacetic acid, Chrzaszcz and Tiukow[13] with malic acid, and Bernhauer and Siebenäuger[5] with succinic acid. However, in the last two papers, the reactants were considered to be produced by mechanisms involving carbon dioxide production. The choice of oxalacetic acid as the four-carbon compound is made more attractive by the modern work on the tricarboxylic acid cycle.

Claisen and Hori,[14] in 1891, were the first to suggest that biological citric acid formation occurred by condensation of acetic acid and oxalacetic acid. Raistrick and Clark[42] postulated this mechanism for the formation of citric acid by molds. Although their mechanism did not involve carbon dioxide production, and yielded 1 molecule of citric acid per molecule of glucose, it was not until the modern work on the tricarboxylic acid cycle in animals and on carbon dioxide fixation[69] that it was recognized the possible mechanism to be the condensation of pyruvic acid and carbon dioxide to give oxalacetic acid, which, in turn, condensed with acetic acid, or a derivative, to yield citric acid. In rough outline, such a series of reactions might be pictured as follows:

Glucose

$\downarrow$

$2\ CH_3{\cdot}CO{\cdot}COOH$

Pyruvic acid

$+\ CO_2 \leftarrow$ $\boxed{CO_2} + CH_3COOH$

Acetic acid

$HOOC{\cdot}CH_2{\cdot}CO{\cdot}COOH$

Oxalacetic acid

$\downarrow$

$$\begin{array}{c} HOOC{\cdot}CH_2{\cdot}C(OH){\cdot}CH_2{\cdot}COOH \\ | \\ COOH \end{array}$$

Citric acid

El-Kerdany,[18] who carried out surface citric acid fermentation in the presence of heavy carbon, $C^{13}$, found heavy carbon chiefly in the terminal carboxyl groups of the citric acid produced although some was present in the secondary carboxyl group. This is con-

sistent with a mechanism involving carbon dioxide uptake, but unfortunately, the ratio between the isotope content of the citric acid and that of the fermentation carbon dioxide was not determined. Martin, Wilson, and Burris,[29] working with mycelial pellets produced in submerged culture, found that in the presence of radioactive carbon dioxide, labeled carbon appeared approximately equally in all carboxyl groups of the citric acid. The amount of labeled carbon incorporated was 0.16 to 0.33 carbon atoms per citric acid molecule. The small amount of incorporation can be explained by the probable greater dilution of the isotopic carbon dioxide in the cell than in the medium. The presence of radioactive carbon in the secondary carboxyl group is explained by the authors, suggesting that the oxalacetate formed from pyruvate by carbon dioxide fixation undergoes reversible conversion to fumarate or other symmetrical compounds.

Weinhouse and Lewis[65] found that carboxyl-labeled acetate was incorporated into citric acid formed by *A. niger*. As in previous experiments with yeast from the same laboratory,[66] it was found that the terminal carboxyls of the citric acid had approximately three-fourths the specific activity of the carboxyl group of added acetic acid, while the secondary carboxyl group had about half the specific activity of the acetate carboxyl. This is a strong indication that the citric acid was formed by condensation of the added acetate with oxalacetate arising from operation of the citric acid cycle. The experiments thus indicate the occurrence of the citric acid cycle in *A. niger*. However, under conditions where citric acid accumulates, the cycle is manifestly not operative. Thus, the experiments were probably performed on mycelium not sufficiently deficient to bring about citric acid accumulation.

If it is concluded from the experiments of Weinhouse and Lewis that a citric acid cycle exists in *A. niger* and from the experiments of El-Kerdany and of Martin *et al.* that oxalacetate arises from carbon dioxide and pyruvate, the only further necessary assumption is that in mycelium sufficiently deficient to accumulate citric acid, the enzymes necessary to metabolize citrate through the cycle or otherwise are only slightly active. In the interpretation of experiments involving carbon dioxide uptake, due consideration must be given to the fact that carbon from carbon dioxide, by equilibration, will often find its way into compounds the formation of which does not involve obligate carbon dioxide uptake. An

example is the accumulation of labeled glycogen in animals in the presence of labeled carbon dioxide. The incorporation of labeled carbon from carbon dioxide into citric acid does not constitute unequivocal evidence that carbon dioxide is a normal intermediate in citric acid formation. However, some mechanism similar to the one sketched before seems to be the most probable mode of citric acid formation in *A. niger.*

## REVIEWS

Many excellent reviews on the citric acid fermentation have appeared. A more complete discussion of some of the points only briefly considered here will be found in a number of them. An earlier review is the paper by Wells and Herrick.[67] Later reviews appear in the publications by von Loesecke,[28] Foster,[19] Perlman,[34] Prescott and Dunn,[39] and Walker.[58]

## BIBLIOGRAPHY


1. Anon., *Chem. Inds. Week,* **68**, 19 (1951).
2. Bernhauer, K., N. Blöckl and H. Siebenäuger, *Biochem. Z.* **253**, 37 (1932).
3. Bernhauer, K., and A. Iglauer, *Biochem. Z.,* **286**, 45 (1936); **287**, 153 (1936).
4. Bernhauer, K., J. Rauch, and G. Gross, *Biochem. Z.,* **319**, 499 (1949).
5. Bernhauer, K., and H. Siebenäuger, *Biochem. Z.,* **240**, 232 (1931).
6. British Intell. Obj. Sub-committee, *Final Rept. No. 220,* Item No. 22, London, H. M. Stationery Office.
7. British Intell. Obj. Sub-committee, *Final Rept. No. 489,* Item No. 22, London, H. M. Stationery Office.
8. British Intell. Obj. Sub-committee, *Final Rept. No. 1176,* Item No. 22, London, H. M. Stationery Office.
9. Buchner, E., and H. Wüstenfeld, *Biochem. Z.,* **17**, 395 (1909).

9a. Buelow, G. H., and M. J. Johnson, *Ind. Eng. Chem.,* **44**, 2945 (1952).

10. Butkevich, V. S., and M. S. Gaevskaya, *Compt. rend. acad. sci. U.S.S.R. (N.S.).* **3**, 405 (1935).
11. Butkevich, V. S., and A. G. Timofeeva, *Biochem. Z.* **275**, 405 (1935).
12. Chrzaszcz, T., and E. Peyros, *Biochem. Z.,* **280**, 325 (1935).
13. Chrzaszcz, T., and D. Tiukow, *Biochem. Z.,* **229**, 343 (1930).

14. Claisen, O. L., and E. Hori, *Ber.*, **24**, 120 (1891).
15. Currie, J. N., *J. Biol. Chem.*, **31**, 15 (1917).
16. Doelger, W. P., and S. C. Prescott, *Ind. Eng. Chem.*, **26**, 1142 (1934).
17. Eisenman, W., and M. D. Blumenfeld, U. S. Patent 2,364,701 (1944).
18. El-Kerdany, M. H., *Fixation of Heavy Carbon Dioxide by Aspergillus niger*, M.S. Thesis, Iowa State College, Ames, (1947).
19. Foster, J. W., *Chemical Activities of Fungi*, New York, Academic Press, 1949.
20. Gerhardt, P., W. Dorrell, and I. L. Baldwin, *J. Bact.*, **52**, 555 (1946).
21. Giordani, M., *Chimica e industria (Milan)*, **17**, 77 (1935).
22. Herzog, R. O., and A. Polotzky, *Z. physiol. Chem.*, **59**, 125 (1909).
23. Karow, E. O., *The Production of Citric Acid in Submerged Culture*, Ph.D. Thesis, Rutgers University, New Brunswick, N. J. (1942).
24. Karow, E. O., and S. A. Waksman, *Ind. Eng. Chem.*, **39**, 821 (1947).
25. Kovats, J., *Przemysl Chem.*, **2**, 122 (1946).
26. Kovats, J., *Gaz. Cukrownicza*, **87**, 142 (1947).
27. Kovats, J., *Gaz. Cukrownicza*, **89**, 30 (1949).
28. Loesecke, H. W. von, *Chem. Eng. News*, **23**, 1952 (1945).
28a. Martin, S. M., and W. R. Waters, *Ind. Eng. Chem.*, **44**, 2229 (1952).
29. Martin, S. M., P. W. Wilson, and R. H. Burris, *Arch. Biochem.*, **26**, 103 (1950).
30. Mazé, P., and A. Perrier, *Compt. rend.*, **139**, 311 (1904).
31. Mazé, P., and A. Perrier, *Ann. inst. Pasteur*, **18**, 553 (1904).
31a. Moyer, A. J., *Bact. Proc.* **1951**, 26.
32. Nakezawa, R., Y. Takeda, and M. Nakano, *J. Agr. Chem. Soc. Japan*, **13**, 52 (1937).
33. Perlman, D., *Citric Acid Production by Aspergillus niger*, M.S. Thesis, University of Wisconsin, Madison (1943).
34. Perlman, D., *Econ. Bot.*, **3**, 360 (1949).
34a. Perlman, D., W. E. Brown, and S. B. Lee, *Ind. Eng. Chem.*, **44**, 1996 (1952).
35. Perlman, D., W. W. Dorrell, and M. J. Johnson, *Arch. Biochem.*, **10**, 131 (1946).
36. Perlman, D., D. A. Kita, and W. H. Peterson, *Arch. Biochem.*, **11**, 123 (1946).

37. Perquin, L. H. C., *Beijdrage tot de kennis der oxydatieve dissimilatie van Aspergillus niger van Tiegham,* Thesis, Technical Hochschule, W. D. Meinema, Delft (1938).
38. Porges, N., *Am. J. Botany,* **19**, 559 (1932).
39. Prescott, S. C., and C. G. Dunn, *Industrial Microbiology,* 2nd ed., New York McGraw-Hill, (1949).
40. Quilico, A., and A. Di Capua, *Giorn. chim. ind. appl.,* **14**, 289 (1932).
41. Quilico, A., and A. Di Capua, *Ann. chim. applicata,* **24**, 355 (1934).
42. Raistrick, H., and H. B. Clark, *Biochem. J.,* **13**, 329 (1919).
43. Raper, K. B., and C. Thom, *A Manual of the Penicillia,* Baltimore, Williams and Wilkins, 1945.
44. Schweiger, L. B., and R. L. Snell, U. S. Patent 2,476,159 (1949).
45. Shu, P., *Ph.D. Thesis,* University of Wisconsin, Madison (1947).
46. Shu, P., and M. J. Johnson, *J. Bact.,* **54**, 161 (1947).
47. Shu, P., and M. J. Johnson, *J. Bact.,* **56**, 577 (1948).
48. Shu, P., and M. J. Johnson, *Ind. Eng. Chem.,* **40**, 1202 (1948).
49. Snell, R. L., and L. B. Schweiger, U. S. Patent 2,492,667 (1949).
50. Szücs, J., U. S. Patent 2,353,771 (1944).
51. Szücs, J., U. S. Patent 2,438,136 (1948).
52. Thom, C., and J. N. Currie, *J. Agr. Research,* **7**, 1 (1916).
53. Tomlinson, N., J. J. R. Campbell, and P. C. Trussel, *J. Bact.,* **59**, 217 (1950); **61**, 17 (1951).
54. U. S. Bureau of Manufacturers, *Monthly Consular and Trade Repts. No. 357, 145* (1910).
55. U. S. Tariff Commission, *Summaries of Tariff Information,* **1**, pt. 1, 13 (1948).
56. Waksman, S. A., U. S. Patent 2,400,143 (1946).
57. Waksman, S. A., and E. O. Karow, U. S. Patent 2,394,031 (1946).
58. Walker, T. K., *Advances in Enzymol.,* **9**, 537 (1949).
59. Wehmer, C., *Compt. rend.,* **117**, 332 (1893).
60. Wehmer, C., *Chem. Centr.,* **1893**, *II*, 457.
61. Wehmer, C., U. S. Patent 515,033 (1894).
62. Wehmer, C., *Chem. Ztg.,* **21**, 1022 (1897).
63. Wehmer, C., *Chem. Ztg.,* **33**, 1281 (1909).
64. Wehmer, C., *Pure Products,* **6**, 317 (1910).
65. Weinhouse, S., and K. F. Lewis, *Abstracts,* Am. Chem. Soc., 116th Meeting, p. 47c (1949).
66. Weinhouse, S., and R. H. Millington, *J. Am. Chem. Soc.,* **69**, 3089 (1947).

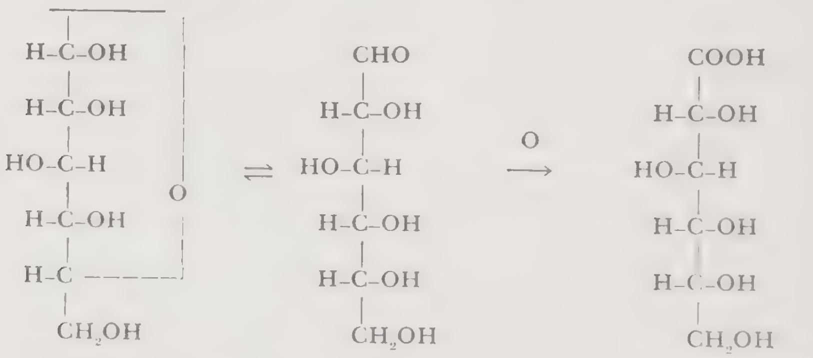
67. Wells, P. A., and H. T. Herrick, *Ind. Eng. Chem.*, **30**, 255 (1938).
68. Wells, P. A., A. J. Moyer, and O. E. May, *J. Am. Chem. Soc.*, **58**, 555 (1936).
69. Wood, H. G., *Physiol. Rev.* **26**, 198 (1946).
70. Woodward, J. C., R. L. Snell, and R. S. Nicholls, U. S. Patent 2,492,673 (1949).

CHAPTER 14

# GLUCONIC ACID

*L. A. Underkofler*

Gluconic acid is the pentahydroxy acid which results from the oxidation of the sugar, glucose:



*Glucose* *Gluconic acid*

Industrial manufacture of gluconic acid is principally for the production of the calcium salt which is used as a pharmaceutical. Recently,[8a] the use of sodium gluconate has been suggested to prevent precipitation of salts from natural waters when caustic soda is added. This might lead to a large market since such use would prevent scale formation in automatic equipment, such as bottle-washing machines.

The production of gluconic acid by microbial oxidation of glucose was first reported by Boutroux,[9] in 1880. He found that a culture of *Acetobacter aceti* (designated *Mycoderma aceti* by Boutroux) produced a nonvolatile acid from glucose which he first thought was lactic acid, but later identified as gluconic acid. In 1887, Boutroux[10] reported that other species of acetic bacteria also produced gluconic acid. Subsequently, many other investigators have reported the production of gluconic acid and ketogluconic acids (Chapter 1 of Volume II) by various species of bacteria and several patents have been granted for the bacterial production of gluconic acid. These will be discussed later.

The production of gluconic acid by mold oxidation was apparently discovered by Molliard[37] and reported in 1922. This acid was produced by a strain of *Aspergillus niger* (designated *Sterigmatocystis nigra* by Molliard) in a medium containing sucrose. Molliard made some attempts to develop optimum conditions for the fermentation.[38]

About the same time, Bernhauer,[2] while studying acid production by *Aspergillus niger,* discovered a strain which produced gluconic acid in high yields when cultivated on glucose in the presence of calcium carbonate. In a series of investigations, Bernhauer[3,4,6] discovered conditions favoring the production of gluconic acid. He reported that media low in nitrogen, producing thin mats and incubated at low temperature, favored the production of gluconic acid, while media high in nitrogen, producing abundant mycelial mats and incubated at relatively high temperature, favored the production of citric acid rather than of gluconic acid. Based on this work, Bernhauer and Schulhof[7] patented a two-stage process. *A. niger* was cultured on a nutrient medium, such as one containing sugar, peptone, monopotassium phosphate, magnesium sulfate, and calcium chloride. The developed mycelial mat was then used for oxidizing an alkaline glucose solution in the absence of nutrient salts and the salt of gluconic acid was recovered.

An extensive study of the gluconic acid fermentation was conducted over a period of years by workers of the United States Department of Agriculture, beginning about 1926. This work will be discussed in some detail since the industrial exploitation of gluconic acid fermentation was based on this research.

## MECHANISM

The conversion of glucose to gluconic acid is a simple oxidation of the aldehyde group of the sugar to a carboxyl group. This change is about as simple a transformation as a microorganism can produce in a hexose. This is probably about the only carbohydrate transformation carried out by fungi which is definitely and fully understood.

Just 3 years after Molliard reported gluconic acid in *A. niger* cultures, Müller[43,44,45,46] discovered a cell-free enzyme preparation which promoted the uptake of molecular oxygen during gluconic acid formation. He named the enzyme glucose-oxidase. Following Müller's work, Franke and coworkers[18,19,20] studied the enzyme in considerable biochemical detail. Müller and Franke obtained and concentrated the enzyme from the press juice of *A. niger* mycelium.

Later, it was discovered[13,26,49] that a powerful antibiotic agent, variously designated as notatin, Penicillin B, and penatin, which was present in culture filtrates from *Penicillium notatum* and other fungi, was identical with the glucose-oxidizing enzyme of Müller.

The enzyme is a flavoprotein[14] which can be resolved into inactive protein and the riboflavin prosthetic group, identified as an alloxazine-adenine dinucleotide by Keilin and Hartree.[29,30] The enzyme apparently is not an oxidase in the classical sense, but is rather a dehydrogenase capable of oxidizing glucose to gluconic acid independently of oxygen, if a suitable hydrogen acceptor is present.[18,19,20] Therefore, the name glucose aerodehydrogenase is now used, denoting dehydrogenation activity with the ability to utilize oxygen as a hydrogen acceptor.

The enzyme promotes the conversion of glucose to gluconic acid, with the simultaneous uptake of one atom of oxygen. One mole of hydrogen peroxide is formed per mole of sugar oxidized.[14,31] The stoichiometric relations for this reaction have been shown to be

$$CH_2OH\cdot(CHOH)_4CHO + H_2O + O_2 \longrightarrow CH_2OH\cdot(CHOH)_4COOH + H_2O_2$$

The formation of the hydrogen peroxide can be demonstrated only with the pure enzyme itself. With living cells, the presence of catalase destroys the hydrogen peroxide, so that the over-all reaction becomes

$$CH_2OH\cdot(CHOH)_4CHO + \tfrac{1}{2}O_2 \longrightarrow CH_2OH\cdot(CHOH)_4COOH$$

The antibacterial action of notatin depends on the presence of

glucose and oxygen; it is the formation of hydrogen peroxide by notatin which accounts for the powerful antibiotic activity of the enzyme.[14,26] The enzyme itself is devoid of antibacterial action.

The glucose aerodehydrogenase is quite specific in its action on glucose.[19,20,30] With a virtually pure enzyme preparation, mannose and xylose were the only other sugars or biological substances oxidized and these at a rate which was only about 1% that of glucose.[14]

A review of the methods of isolation, properties, and mechanism of action of the glucose aerodehydrogenase is given by Foster.[17]

## BACTERIAL PRODUCTION OF GLUCONIC ACID

Since the time of the discovery of gluconic acid production by acetic acid bacteria by Boutroux,[9,10] many investigators have reported the production of this acid by other species of bacteria. A majority of the species of the genus *Acetobacter* apparently form this acid from glucose, but usually continue the oxidation further with the production of ketogluconic acids. *Pseudomonas* species also produce gluconic acid from glucose as an intermediate in the formation of 2-ketogluconic acid (Chapter 1, Volume II). Butlin[12] has reviewed the literature on the production of gluconic acid by acetic acid bacteria. Among those listed by Butlin are: *A. aceti,*[9,11,23,54] *A. acetosum,*[23] *A. ascendens,*[23] *A. gluconicum,*[22,23] *A. Hoshigaki* var. *rosea,*[52] *A. industrianum* var. *Hoshigaki,*[53] *A. kutzingianum,*[23] *A. orleanense,*[23] *A. pasteurianum,*[10,23] *A. rancens,*[23,56] *A. suboxydans,*[56] *A. xylinoides,*[23] and *A. xylinum.*[6,8,11,23] Recently Bernhauer and Riedl-Tumova[5] have reported rapid oxidation of glucose to gluconic acid and then ketogluconic acids by *A. suboxydans muciparum* and *A. melanogenum.* They also found both organisms quickly oxidized D-xylose and L-arabinose to the corresponding pentonic acids, without further oxidation to keto acids. L-Xylose was not oxidized by either organism, while D-arabinose was fermented to a considerable degree by *A. melanogenum.*

Several patents have been issued covering processes for production of gluconic acid by species of acetic acid bacteria. Currie and Carter[15] employed *A. oxydans* to oxidize a glucose solution in the form of thin films in contact with air. A tower similar to a vinegar generator was used, filled with an inert packing, such as wood shavings, coke or clay shards. According to the patent, nutrients, in the form of 0.2 to 2% mineral salts, were added to

the mash; and 25% glucose was stated to be the optimum concentration although concentrations as high as 45% could be employed. A temperature range of 15° to 35°C was given. Hermann,[22] in an Austrian patent, recommended the procedure and apparatus ordinarily used in the quick-vinegar process for fermentation of solutions of glucose with bacteria such as *A. gluconicum.*

Currie and Finlay,[15a] in 1933, patented a process for gluconic acid production by oxidizing glucose with *A. gluconicum* or other bacteria of the *Acetobacter* group in a deep tank with aeration and high speed agitation. Within 48 to 60 hours, 90 to 95% of the glucose in 15% glucose medium was converted to gluconic acid at a temperature of 30° to 34°C. This is certainly among the first recorded applications of the modern method for submerged culturing technique for aerobic organisms.

Takahashi[51] patented a process for production of gluconic acid by fermenting glucose or mannitol solutions with cultures identified as *A. Hoshigaki* var. *rosea* and *A. industrianum* var. *Hoshigaki.* Nitrogen was supplied by addition of soybeans or extract of rice bran. Fermentation for 18 days at 26° to 28°C resulted in yields of gluconic acid up to 103%, based on glucose.

Closely related to gluconic acid production is the patent of Lockwood,[32] covering the production of pentonic acids from pentoses such as arabinose, ribose, and xylose. This process involves fermentation of the media containing the sugar, magnesium sulfate, monopotassium phosphate, calcium carbonate, and a nitrogenous nutrient, such as yeast extract, corn steep liquor, or liver extract, with any of a number of *Pseudomonas* species for about 7 days under aerated conditions. A similar patent by Lockwood and Stodola[33] relates to the preparation of bionic acids, such as lactobionic and maltobionic acids, directly from the disaccharides by *Pseudomonas* fermentations.

So far as can be determined bacterial oxidation of glucose to gluconic acid has not achieved industrial application.

## PRODUCTION OF GLUCONIC ACID BY MOLDS

### Surface Fermentation

Although gluconic acid had previously been reported as a product of mold metabolism, as mentioned before, the development of a successful process in the United States for the production of

this acid by fermentation was the result of intensive work by investigators of the United States Department of Agriculture, starting about 1926.

May, Herrick, Thom, and Church[36] first reported in 1927 the production of gluconic acid by molds of the *Penicillium luteum-purpurogenum* group. Herrick and May[25] reported optimum conditions which they had determined for the production of gluconic acid by this organism in surface culture, using shallow pans. Maximum yields of 55 to 65% of theoretical were obtained. Using these conditions, May, Herrick, Moyer, and Hellbach[34] produced gluconic acid on a semiplant scale in 1929 by fermentation of glucose medium in aluminum pans of $43 \times 43 \times 2$ in., with *P. luteum-purpurogenum.*

An investigation of a considerable number of *Penicillium* species led to the discovery, in 1936, by Moyer, May, and Herrick[40] of a culture of *P. chrysogenum* which had greater capacity for producing gluconic acid and at the same time had biochemical and vegetative vigor superior to those of *P. luteum-purpurogenum* employed previously. Using this culture of *P. chrysogenum,* fermentation in shallow aluminum pans with surface-volume ratio of 0.4 to 0.5 for 8 to 10 days at 30°C gave 60% conversion of glucose to gluconic acid. The medium contained 200 to 250 g commercial glucose, 3.00 g $NaNO_3$, 0.30 g $KH_2PO_4$, and 0.25 g $MgSO_4 \cdot 7H_2O$ per l.

## Submerged Fermentation

In 1928, Schreyer[50] reported the results of a detailed investigation of *A. fumaricus* which produced mixtures of gluconic and citric acids from glucose. Aeration and agitation of cultures to which calcium carbonate had been added caused a four- to six-fold increase in gluconic acid production, but had no effect on citric acid formation. Thies[55] obtained similar results with the same mold by bubbling oxygen instead of air through culture solutions containing calcium carbonate. In a patent of 1933, Currie, Kane, and Finlay[16] claimed yields by gluconic acid forming molds, such as *Aspergillus niger* and *Penicillium luteum,* as high as 90% of theory in 48 to 60 hours. In this process, the modern submerged-culture technique was employed; the culture liquid was maintained in a high state of agitation by means of a high-speed stirrer, at the same time drawing large volumes of air into the solution.

In 1934, May, Herrick, Moyer, and Wells[35] investigated pro-

duction of gluconic acid by *P. chrysogenum* when cultivated in submerged culture. They employed washing bottles with sintered-glass false bottoms under increased air pressure. The bottles were incubated in a pressure vessel and yields of gluconic acid of 80 to 87% were obtained in 8 days from 20% glucose medium at 30°C at an air pressure of 3 atm or more.

In an attempt to devise an apparatus for culture of molds in larger quantities under increased air pressure, Herrick, Hellbach, and May[24] designed a rotary-drum fermentor in 1935. Buckets and baffles on the inside of the drum served to bring the oxygen of the air into intimate contact with the medium and the submerged mycelium as the drum was rotated. The first laboratory drum had a diameter of 9 in. and was 12 in. long. With this drum, at 30 psi air pressure, yields of gluconic acid approaching 80% of theory were obtained in 56 hours as compared with 8 days for similar yields in the previous glass apparatus.

Continued work with the drum by Wells, Moyer, Stubbs, Herrick, and May[58] led to the conclusion that *P. chrysogenum* would not be a satisfactory organism for commercial-scale operations, because it does not produce the large quantities of spores necessary for inoculation. Another organism, *Aspergillus niger,* strain 67, was, therefore, selected for further work since it produced spores in abundance and gave uniform fermentation. These workers made a detailed investigation of the optimum cultural conditions in the laboratory drums and achieved gluconic acid yields of 84% from 15% glucose medium in an 18-hour fermentation period.

In 1937, Moyer, Wells, Stubbs, Herrick, and May[42] reported further results of their laboratory investigations on methods for inoculum development and optimum composition of fermentation medium for gluconic acid production by submerged cultivation of *A. niger,* strain 67, under superatmospheric pressures. Using a medium containing, per liter, 100 to 150 g glucose, 0.156 g $MgSO_4 \cdot 7H_2O$, 0.188 g $KH_2PO_4$, 0.388 g $(NH_4)_2HPO_4$ and 26.0 g $CaCO_3$, yields up to 95% of theory were obtained.

The success of the laboratory work led to the design of a large aluminum drum, 3 ft in diameter by 9 ft total length, for pilot-plant work. This drum was first described in 1937 by Wells, Lynch, Herrick, and May,[57] and pilot-plant-scale operations with this drum were reported by Gastrock, Porges, Wells, and Moyer.[21] Medium composition, inoculum development, neutralizing agent, rate of

rotation, rate of air flow, glucose concentration, volume of fermentation medium and reuse of the fungus mycelium were investigated. With a medium containing 150 to 200 g glucose and 26 g calcium carbonate per l, consistent yields of at least 95% were obtained in 24-hour fermentations in the large drum, using 140 gal of medium. Optimum conversions were obtained with an air pressure of 30 psi, air flow of 375 ml per l of medium per minute and a rotation speed of 9.5 or 11.8 rpm.

Porges, Clark, and Gastrock[48] developed the process for semi-continuous operation. This involved reuse of the mold mycelia, and was studied in both laboratory-size and large pilot-plant drums. Two methods were investigated: (1) transferal of an aliquot of the actively fermenting solution with its proportionate amount of fungal growth to a second drum, containing freshly prepared medium, and (2) flotation of the mycelium by reducing the pressure to atmospheric in the resting drum, drawing off the lower 80% of the completely fermented charge, and introducing a new charge. Both procedures were successful in lowering fermentation time, but the flotation procedure gave best results and fermentation was usually complete in about 9 hours. With this procedure, it was found that for optimum results, the glucose concentration should be reduced to about 11.5 g from the 15 g per 100 ml used previously. As many as fifteen successive fermentations were conducted in this manner with no apparent loss in effciency.

About a year later, Porges, Clark, and Aronovsky[47] reported that the mycelium of *A. niger,* strain 67, could be recovered by pressure filtration in a sterile aluminum filter or by centrifugation and reused for inoculation of fresh sterile fermentation medium. The amounts of mycelium returned to the rotary fermentor for each succeeding fermentation averaged 75% of the amounts recovered from the preceding one. With this method, 16 g per 100 ml of glucose could be used successfully, and as many as nine successive fermentations were conducted. The advantage of this procedure was that it made possible the use of a full charge of fresh medium of higher glucose concentration than was feasible with the flotation method previously reported.

It is, of course, generally desirable to conduct fermentations at as high substrate concentrations as possible to facilitate the recovery of product and to reduce plant costs. The previously described gluconic acid fermentations were limited to a maximum of about

16% glucose because of inhibition of fermentation by precipitation of calcium gluconate or through injury to the fermenting organism by exposure to free gluconic acid.

In 1940, Moyer, Umberger, and Stubbs[41] found it possible to ferment solutions containing up to 35% glucose by addition of boron compounds which temporarily stabilized the solution and prevented precipitation of calcium gluconate. Under these conditions, an excess of calcium carbonate could be used to neutralize all of the gluconic acid formed. This eliminated the decrease in fermentation rate during the final stage of fermentation and the long lag phase when preformed mycelium was used to ferment a new charge of glucose medium. It was found that precipitation of calcium gluconate during normal fermentation of 20, 25, 30, and 35 g glucose per 100 ml can be prevented by 500, 1,000, 1,500, and 2,500 ppm of boron, respectively. However, it was also found that the strain of mold employed in former studies, *A. niger* 67, did not grow well in the presence of more than 400 ppm of boron. However, another strain, *A. niger* 3, produced gluconic acid well and growth was not inhibited at 2,000 ppm of boron, the tolerance being even higher in the presence of calcium carbonate. With this organism, using the fermentation medium previously employed,[42] except that the concentration of diammonium phosphate was increased to 0.5 g per l and 2 to 3 ml of corn steep liquor was added per liter, excellent fermentations were secured. In these fermentations, excess calcium carbonate and 1,500 ppm of boron were used with 25% glucose medium. The boron was best added after the fermentation was well under way and in the form of dry boric acid or borax in order to prevent violent evolution of carbon dioxide, foaming of the medium, and excessive increase in volume. By reuse of the mycelium filtered from a previous fermentation, it was possible in the laboratory drums to ferment 25% glucose solutions to gluconic acid in a 24-hour cycle. Four successive fermentations with reuse of mycelium gave practically identical fermentation curves, with about 95% conversion efficiency. The use of boron compounds in the gluconic acid fermentation was patented by Moyer.[39]

## THE COMMERCIAL FERMENTATION

### Cultures and Their Maintenance

Apparently, *Aspergillus niger* is the only organism which has

been employed on industrial scale for gluconic acid production. Strains of this organism must be selected which give gluconic acid with exclusion of other acids, particularly citric and oxalic acids. Strains resistant to the action of boron must be employed if boron compounds are to be used in the fermentation. Cultures may be developed in the laboratory on slants of agar medium, and may be maintained as soil cultures or by lyophilization (Chapter 12, Volume II). Blom *et al.*[8a] have recently recommended to carry stock cultures of *A. niger* NRRL–3 on agar slants of the following composition: 30 g glucose, 0.10 g $MgSO_4 \cdot 7H_2O$, 0.12 g $KH_2PO_4$, 0.25 g $NH_4NO_3$, 0.25 g peptone, 4.00 g $CaCO_3$, 25.0 g agar, aqueous extract from 200 g of peeled, sliced, and cooked potatoes, tap water to make 1,000 ml. The potato extract is prepared by autoclaving 200 g of peeled and sliced potatoes in 500 ml of tap water for 15 minutes and filtering through cheesecloth.

## Development of Cultures to Plant Stage

Generally, gluconic acid fermentations in industry employ reuse of mycelium and once the process is operating properly, there is little need to build up cultures through laboratory and plant culture tanks. However, such a procedure is necessary in initially starting up the plant operations and in case of contamination of plant fermentors, requiring the use of a new culture. The procedure recommended by Moyer, Wells, Stubbs, Herrick, and May[42] involves pregermination of the mold spores. However, this can be eliminated by initially inoculating the plant fermentors with spores developed in the laboratory. Spore cultures have been prepared for large-scale laboratory gluconic acid fermentations by the author by growing the mold on moist bran in the manner used for spore-inoculum development for fungal enzyme production (Chapter 3, Volume II). Blom *et al.*[8a] recommended inoculum spore production by laboratory culturing of the mold for 7 days on 150 ml portions of medium containing, per 1,000 ml, 50.0 g glucose, 0.12 g $MgSO_4 \cdot 7H_2O$, 0.15 g $KH_2PO_4$, 0.20 g KCl, 0.60 g $(NH_4)_2HPO_4$, 0.01 g ferric tartrate, 30 ml potato extract (prepared as described before), 30 ml beer, and 1.5 g agar. Moyer *et al.*[42] employed for spore germination surface culturing of the mold on a liquid medium containing, per liter, 91.5 g glucose, 0.450 g $NH_4NO_3$, 0.072 g $KH_2PO_4$, 0.060 g $MgSO_4 \cdot 7H_2O$, and 60 ml of beer. Blom *et al.*[8a] prepared vegetative growth inoculum for 150-gal pilot-plant fermentation in

45 gal of medium containing 10 kg glucose, 750 ml corn steep liquor, 31 g $MgSO_4 \cdot 7H_2O$, 38 g $KH_2PO_4$, 20 g urea, 80 g $(NH_4)_2HPO_4$, 25 g NaOH to bring pH to 6.5, and tap water to make 45 gal after sterilization for 30 minutes at 121°C. The medium was inoculated with two flasks of spore culture prepared as described previously and incubated for 24 hours at 33°C with aeration at the rate of one-fifth volume per minute per volume of medium.

## Contamination Problems

Contamination is not a serious problem in this fermentation. The fermentations are rapid and contaminants have little chance to develop. Likewise, the medium employed is inadequate for most organisms, such as acetic acid bacteria, which might be contaminants. Wells, Moyer, Stubbs, Herrick, and May[58] even ventured the opinion that: "It may thus be possible to carry out the fermentation on a large scale without sterilization of either equipment or the nutrient solution." Such practice is not generally recommended, however. Reuse of filtered mycelium for inoculation of successive fermentations reported by Gastrock *et al.*[21] and by Moyer, Umberger, and Stubbs[41] employed filtered mycelium which had opportunities for contamination by bacteria and other fungi. Examination of the fermented medium after four cycles by the latter authors revealed no contamination, and they stated: "Apparently this fermentation is not very susceptible to contamination by most common bacteria and fungi."

## The Plant Fermentation

No detailed description of commercial operation of the gluconic acid fermentation has been published. However, it is well known that the procedures used are based on the developments made by the investigators of the United States Department of Agriculture discussed before. Selected strains of *A. niger* are employed; the fermentation is conducted by submerged culture of the mold with aeration under pressure; the mold mycelium is reused for successive fermentations. The fermentations may be run in rotary drums or in conventional vat fermentors, equipped with porous stones or perforated pipes for the dispersal of air. The fermentor should be constructed of enameled steel, or preferably of high-purity aluminum or nickel-free stainless steel. Mechanical agitation is advantageous and probably essential for a rapid cycle. During fermentation,

vigorous aeration with sterile air must be provided. Fermentation under air pressure of 2 atm (30 psi gage pressure) results in more rapid operation. The volume of air supplied will depend on the efficiency with which the solution is aerated and the air requirement will be less if mechanical agitation and fermentation under super-atmospheric pressure are used. The preferred temperature is 30°C. Considerable heat is evolved during the fermentation, and cooling is necessary in large-scale fermentations. Internal coils and external water jackets or sprinkler systems have been found satisfactory for temperature control.

A suitable production medium contains, per liter, 100 to 150 g glucose, 0.156 g $MgSO_4 \cdot 7H_2O$, 0.188 g $KH_2PO_4$, 0.388 g $(NH_4)_2HPO_4$, and 26.0 g $CaCO_3$, the last being preferably sterilized separately from the other ingredients. The medium is sterilized in a separate vessel and transferred aseptically to the fermentor in which the mold mycelium from a previous fermentation has been retained. Glucose solutions of 25% glucose concentration, or even higher, may be employed, if excess calcium carbonate is added along with about 1,500 ppm of boron as dry boric acid or borax after the fermentation is well under way. Octadecanol has been successfully used as an antifoam agent.

### British Process

Apparently the only publication dealing with commercial procedures for the gluconic acid fermentation is the general description of British practice by Williams.[59] The process employs semicontinuous fermentation with reuse of mold mycelium and quite evidently is based on the drum process developed in the United States as previously discussed.

**Medium Preparation.** The fermentation medium is prepared in a batch-mixing and sterilizing vessel, which is a vertical, cylindrical, closed vessel with conical bottom and domed top. It is made of steel with all interior parts covered with enamel. The vessel is fitted with a stirrer shaped and adjusted to just clear the conical bottom. An aluminum coil of large surface area is fitted internally which is connected with steam and cold water for the rapid heating or cooling of the batch as desired. The domed top has a hinged charging door which can be closed by screw clamps and made airtight. Reduced pressure may be applied by vacuum pump to the mixer through a large pipe in the dome. In the base of the vessel

is an aluminum air line for passage of air upward through the batch. The air line passes horizontally outside the vessel where it is connected with a perpendicular pipe, the uppermost end of which is higher than the height of the liquid batch in the mixer. The air line is attached to an air filter.

In operation, the raw materials are charged into the mixer through the manhole in the top. A batch of convenient size will contain 300 lb of glucose and 300 to 360 gal of hot water. The agitator is then started, and steam turned into the coil. When the glucose has been completely dissolved, several per cent, on the weight of sugar, of nutrient salts are added, these salts generally being various boron and ammonium compounds. Powdered calcium carbonate is then added. The amount of calcium carbonate used should not be sufficient to neutralize all of the gluconic acid which will be formed during fermentation, for the presence of some free acid at the end facilitates separation of mycelium for reuse in the next batch. Approximately 1 g of the carbonate will neutralize the gluconic acid from 3.6 g of glucose. Consequently, 60 to 70 lb of calcium carbonate would normally be added for a batch containing 300 lb of glucose.

The mixer is closed and heating is continued until boiling begins, then the steam flow is reduced to maintain a steady boiling temperature. During the boiling, filtered air is drawn upward through the pipe in the base of the vessel, the air and water vapor being drawn off through the vacuum system. Boiling is continued for about an hour, or until the mixer contents are judged to be thoroughly sterilized. The steam and air are then shut off and cooling water is passed through the coil until the temperature of the stirring mash has been reduced to 30° to 32°C, the vacuum pump continuing to draw on the vessel as cooling proceeds, thus giving a relatively high vacuum when the mixer contents become cool. When the desired temperature has been reached, the vacuum is released and the mixture is passed into the fermentor. With an average installation, 8 to 10% of the water originally added is evaporated during the boiling and cooling and the glucose content of the finished mash will be 10 to 13%.

**Fermentation.** The fermentor is conveniently situated immediately below the batch-mixing vessel, so that sterilized mash can be run into it directly from the mixer. The type of fermentor commonly used consists of a rotating steel cylinder, enamelled in-

side, mounted on a horizontal axis, the weight of the fermentor being taken by rollers which contact steel tires welded to the shell. The vessel is geared to rotate at 9 to 12 rpm. The internal temperature of the fermentor is controlled through a series of pipes arranged lengthwise in the cylinder and connected to low-pressure steam and cold-water supplies. A perforated air line also runs lengthwise through the vessel. The charging and discharging of the fermentor take place through openings in the ends of the cylinder. Both apertures are near the cylinder surface and so positioned that when the discharge outlet is at the lowest point ready for the fermented batch to be run off, the inlet is at the highest point and ready to receive the next batch of medium from the mixer.

To start a series of fermentations, about 5% by volume of pregerminated culture of *A. niger* is added to the batch in the fermentor. This is 15 to 20 gal of culture. The drum is then set in motion and a current of air is blown into the batch as it rotates. The air stream is supplied at 30 psi gage pressure. The temperature of the fermentor contents is maintained at around 30°C and the progress of the fermentation is followed by periodic sampling and sugar determinations. When the sugar content has fallen below 1%, it is usually uneconomical to carry the process further, for fermentation of the remaining sugar takes place very slowly.

In samples taken from the fermentor before all the calcium carbonate has reacted with the gluconic acid being formed, the mycelium separates from the solution very slowly. But when all the calcium carbonate has been consumed and free gluconic acid is present, the mycelium separates easily by floating to the surface. Apart from ascertaining the sugar content as the fermentation nears the end, a good guide to the progress is also afforded by following the pH which drops near the end of the run. When the fermentation is judged to be complete, the drum is stopped and allowed to remain stationary for 30 to 40 minutes during which time practically all the mycelial growth rises to the surface, forming, as a general rule, 10 to 12% by volume of the batch. The upper layer contains also some calcium gluconate and free gluconic acid.

As soon as there is a clear separation between the mycelium and the solution, the latter is drawn off at the bottom of the fermentor, leaving the mycelium in the vessel ready for addition of the next batch of medium from the mixer-sterilizer. Subsequent fermentations are more rapid than the first one and normally

require 8 or 9 hours. The record for an average batch is shown in Table 51.

Table 51. RESULTS OF A TYPICAL GLUCONIC ACID FERMENTATION

| | |
|---|---|
| Weight of glucose | 300 lb |
| Water | 3,000 lb |
| Solution | 3,300 lb |
| Water evaporated in mixer | 276 lb |
| Weight of sterilized batch | 3,024 lb |
| Sugar concentration at start | 9.92% |
| Fermentation started | 8:20 A.M. |
| Fermentation stopped | 4:45 P.M. |
| Fermentation period | 8 hours, 25 minutes |
| Residual glucose in batch leaving fermentor | 0.75 g per 100 ml |

Pilot-Plant Production of Sodium Gluconate

Recently Blom *et al.*[8a] have published a procedure for large pilot-plant-scale production of sodium gluconate by fermentation with *A. niger* NRRL-3. The stainless-steel fermentor was 13 ft tall with an internal diameter of 24 in. Agitation was provided by a propeller-type blade attached to a horizontal shaft mounted 15 in. above the bottom of the fermentor. The temperature was controlled to within 0.5°F by circulation of water through an external jacket, with automatic control. Most fermentations were conducted at a volume of 150 gal, corresponding to a liquid depth of about 7 ft. Air for the fermentation, sterilized by passing it through a 10-in. column containing 8 ft of 10- to 24-mesh activated carbon, was introduced through a perforated pipe-cross sparger.

Sodium hydroxide for continuously neutralizing the gluconic acid as formed was mixed in a 40-gal stainless-steel supply tank. The pH of the fermenting medium was recorded and automatically controlled. The medium was circulated from the bottom of the fermentor through an enameled-iron flow chamber and pH electrode assembly and back into the top of the fermentor. Alkali solution from the supply tank was added automatically to control the pH at a predetermined constant value. The controller incorporated throttling-range and droop-correction adjustments so that effective pH control was obtained.

Foaming of the medium during fermentation was controlled by automatic addition of antifoam agents. When the foam reached

a predetermined level, it contacted an insulated electrode and activated an electronic controller that opened a solenoid valve in the antifoam supply line.

Variables in the process, including type of antifoam agent, method of sterilization, type of inoculum, agitation, air pressure and aeration rate, sugar concentration, and depth of medium were investigated thoroughly to determine optimum operating conditions. The most satisfactory antifoam agent proved to be 1% octadecyl alcohol in ethyl alcohol. Both continuous sterilization at pH 4.5 in the range of 220°F for 3 minutes and of 275°F for 5 minutes and batch sterilization at pH 4.5 at 250°F (15 psi gage) for 10 to 30 minutes were found suitable. Less antifoam agent was required in fermentations in which batch sterilization was employed, probably due to alteration of nitrogen compounds by the long total heating period necessary in bringing the medium up to sterilization temperature and down to fermentation temperature. Use of spore inoculum required a germination lag period of 10 to 12 hours. Use of vegetative inoculum, varying from 5 to 30% of the final volume, with 10% being sufficient for rapid fermentation, resulted in a lag period of 4 to 6 hours. When mycelium from a good fermentation was reused as inoculum for sterilized medium, the lag period was entirely eliminated and sugar was utilized immediately at high rate.

Increase in any of the operating variables of agitation, fermentation pressure, and aeration rate resulted in increase of rate of sugar utilization. For maximum production rate of gluconate, agitation, fermentation pressure, and aeration were maintained as high as possible. With decrease in fermentation pressure or increase in agitation or aeration rate, more foaming was encountered and thus more antifoam agent was required. For the fermentor employed, optimum conditions were determined to be: aeration, 1.5 volumes per minute per volume of medium; agitation, 220 rpm; fermentation pressure, 30 psi gage.

From results with sugar concentrations varying from 22 to 35%, it was concluded that maximum daily production of sodium gluconate per fermentor would result from a medium containing 30% glucose.

**Recommended Process.** From the experimental results, Blom *et al.*[8a] concluded that sodium gluconate can be produced successfully by continuous neutralization of gluconic acid, as it is formed

in submerged-culture fermentation of glucose by *A. niger* NRRL-3, and recommend the following process.

The inoculated medium should contain 24 to 30% corn sugar (glucose), 0.4% corn steep liquor, 0.02% $MgSO_4 \cdot 7H_2O$, 0.02% $KH_2PO_4$, 0.01% urea, and 0.04% $(NH_4)_2SO_4$. The medium should be sterilized at pH 4.5, preferably batchwise at 250°F for 15 to 30 minutes.

Inoculum for the fermentation should be prepared from a medium containing 6% corn sugar, with other nutrients as listed, batch sterilized at 250°F for 15 to 30 minutes at pH 6.5. Sterile medium should be cooled to 92°F and inoculated with one flask of sporulated culture (110 sq cm of mycelial mat) per 45 gal of seed medium. The seed medium should be aerated with one-fifth volume of sterile air per minute per volume of medium for 24 hours. At the end of this period, the glucose content should be between 4.5 and 5.0% and the pH should be approximately 4.8.

The sterilized production medium should be adjusted to a pH of 6.5 with sodium hydroxide before inoculation and inoculated with 10% by volume of vegetative inoculum. Vigorous mechanical agitation should be employed and finely dispersed sterile air should be passed through the medium at the rate of at least one volume of air per minute per volume of medium. The fermentation should be conducted at a pressure of 20 to 30 psi gage to give a high rate of sugar utilization and to minimize foaming, which should be controlled by addition of 1% octadecyl alcohol in ethanol solution as required. The fermentation temperature should be maintained at 92° to 94°F and the pH should be controlled automatically at $6.5 \pm 0.5$ by addition of strong sodium hydroxide solution. After a lag period of 5 to 7 hours, oxidation of sugar begins, and the sugar should be consumed at a rate of 1.5% per hour until only 0.1% remains.

Subsequent batches of sterile fermentation media may be inoculated with mycelium filtered from the fermented liquor. In this case, there is no lag period and sugar-utilization rate should be approximately 1.5% per hour.

The liquor from a fermentation should be filtered and the mycelium cake discarded or used as inoculum in a following fermentation. The filtrate should then be processed for recovery of the sodium gluconate.

## ANALYTICAL METHODS

The glucose content of the mashes and fermentation samples is determined by conventional sugar-analysis procedures, involving copper reduction. Sugar analysis gives the original concentration of glucose in the mash and the course of the fermentation is followed by sugar analyses on samples taken periodically during the fermentation period. The fermentation is judged complete when residual glucose reaches a predetermined low level.

The course of the fermentation can also be followed conveniently by periodic pH determinations. Until free acid develops, in calcium gluconate fermentations, the pH of the medium remains close to 5.5. With formation of free gluconic acid, the pH drops to 3.5 or even lower.

It is frequently useful to estimate the amount of free gluconic acid in the fermented batch leaving the fermentor. If the amount of gluconic acid present as calcium gluconate is desired, the calcium is determined on filtered liquor by oxalic acid precipitation, followed by permanganate titration.

## RECOVERY

The most common practice is to recover the product as the calcium salt. The fermented liquor is filtered and is mixed, with vigorous agitation, with a suspension of calcium hydroxide calculated to neutralize 98% of the free gluconic acid present. The mixture is cooled below 20°C and allowed to stand for 24 to 48 hours for crystallization. The calcium gluconate crystals are separated from the mother liquor in a centrifuge and washed twice with cold water. The calcium gluconate cake is then removed from the centrifuge and dried in a tray drier at a temperature below 80°C. The mother liquor and washings are concentrated by vacuum evaporation and cooled to obtain a second crop of calcium gluconate crystals.

To obtain free gluconic acid from the fermented beer, sulfuric acid equivalent to the amount of calcium present is added, the calcium sulfate is filtered, and the whole of the acid concentrated to a sirup of the required acid strength by vacuum evaporation. The acid of commerce is a 50% aqueous solution. The delta lactone is also of commercial interest. Conversion of the acid to the delta lactone results on heating or by azeotropic distillation

with butanol or dioxane, and the lactone is crystallized from an anhydrous solvent.

For recovery of sodium gluconate from fermentations neutralized with sodium hydroxide, Blom *et al.*[8a] recommend evaporation of the filtered liquor in a multiple-effect evaporator to a sirup containing 42 to 45% solids, adjustment of the pH to 7.5 with sodium hydroxide, and drum drying to solid, crude sodium gluconate. The drum-dried product should be ground to 100 mesh in a hammer mill for bulk storage and this crude sodium gluconate may be used for water conditioning. If a lighter-colored product is desired, the evaporated liquor may be carbon treated before drum drying. The drum-dried product is light tan in color, 95% pure, and readily soluble in water. Pure-white sodium gluconate may be obtained by crystallization of evaporated fermentation liquor and recrystallization, or by recrystallization of the drum-dried product from alcohol or water.

## RESIDUES AND WASTES

Since the industrial gluconic acid fermentation is carried out on relatively small scale, disposal of the residues and wastes, consisting of filtered mold mycelium and mother liquors from crystallization, has not been reported as a serious problem.

## USES

A recent publication[48a] reviewed the properties of gluconic acid and its derivatives, and listed some of their many present and potential uses. These compounds have become important in the pharmaceutical, food, feed, and general industrial chemical fields.

Gluconic acid has had its chief importance in the pharmaceutical industry in the form of its salts for the purpose of introducing appropriate metallic ions, such as calcium, iron, and potassium, into the body in a neutral, nontoxic, and easily assimilable form. Calcium gluconate is the preferred calcium carrier for supplying this metal in cases of calcium deficiency in children and to women in pregnancy. Calcium gluconate has been successfully employed in human medicine during the past 20 years in the treatment of tetany and other calcium deficiencies. In veterinary practice, injection of calcium gluconate into milch cows suffering

from milk fever has given very good results. Calcium gluconate is a satisfactory source of calcium for poultry; the quality of egg shells is improved when it is included in the ration.

Ferrous gluconate, because of its nonirritating properties, is an excellent source of iron in the treatment of nutritional anemia and for iron therapy. Recently, Bernhard[1] obtained excellent results in the use of potassium gluconate as a source of potassium in hypopotassemia in medical and surgical practice. Stone[50a] stated that the salts of gluconic acid serve as an efficient means of introducing trace elements into the diet.

Free gluconic acid and its lactone have numerous uses. They have applications as chemical reagents for preparation of lower sugars as well as esters and other carbohydrate derivatives. Present and proposed uses in foods include the addition of small amounts of gluconic acid to shortenings to give more stable products less likely to become rancid and use of glucono-δ-lactone in baking powders, since it causes more gradual liberation of carbon dioxide than is possible with tartaric acid salts. Gluconic acid is widely used in the dairy industry to prevent formation of milkstone, and recently it has been shown to be effective in the prevention of beerstone in breweries.

In the textile industry, gluconic acid, glucono-δ-lactone, and ammonium gluconate are employed as acid catalysts. For example, the requirement for an odorless, nontoxic acid catalyst for acid colloid resins used with textiles has led to wide acceptance of gluconic acid or its lactone for this purpose. Recently, sulfonated gluconamides produced by reaction of glucono-δ-lactone with fatty acid amines have been described as effective detergents.[36a]

A possible large-scale use for sodium gluconate is in preventing precipitation of calcium and magnesium salts from natural hard waters, in applications requiring caustic solutions.[8a,48a] Caustic-soda solutions have extensive uses in industry. Prevention of insoluble hard-water precipitates by sodium gluconate addition occurs over a wide range of caustic-soda concentrations. The quantity of sodium gluconate required varies with the degree of hardness, but sodium gluconate amounting to 5 or 10% of the weight of caustic soda appears to be sufficient for waters usually encountered. This application should have commercial significance in automatic equipment, such as bottle-washing machines used by

the dairy, carbonated beverage and brewing industries. Another possible application is in the textile industry, where the use of sodium gluconate might prevent deposition of hard-water salts on the fabric, thus obviating need for their subsequent removal.

The possible use of sodium gluconate as a metal sequestering agent in solutions of high alkalinity prompted the pilot-plant research reported by Blom *et al.*[8a] for developing the sodium gluconate fermentation process which has been described before. During the developmental work, over 3,000 lb of sodium gluconate were recovered and this compound is under test in several industrial installations to determine its effectiveness where prevention of scale or hard-water precipitates is desired. Blom *et al.*[8a] have estimated that a plant to produce 3 million pounds of sodium gluconate annually would require an investment of $390,000. They have estimated that plant-production costs for manufacturing 9,610 lb of sodium gluconate a day, or 3,000,000 lb a year, would be 11.78¢ per pound of gluconate produced with corn sugar at 7.5¢ per pound. These plant-production costs do not include administrative and selling expenses.

## COMPETITIVE OPERATIONS AND PROCESSES

Besides its production by fermentation, gluconic acid may be manufactured by chemical oxidation of glucose. The most feasible chemical method for obtaining aldonic acids from the corresponding aldose sugars apparently involves the electrolysis between carbon electrodes of solutions containing sugars, small amounts of bromides, and a buffer, such as calcium carbonate. This process proved to be very satisfactory for the manufacture of calcium gluconate in considerable quantities.[28] The reaction apparently takes place by the formation of free bromine at the anode; the bromine oxidizes the aldose to aldonic acid and is reduced to bromide. The principal products of the reaction, when glucose is employed, are calcium gluconate, carbon dioxide, and hydrogen. The calcium gluconate which crystallizes from the electrolyte is collected on a filter and the mother liquors containing the bromide are returned to the cell after the addition of more glucose and calcium carbonate. Yields are said to be almost theoretical in many instances. The process has been patented by Isbell.[27] A disadvantage is that if the electrolytic method is not well controlled, saccharic acids and 2-keto- and 5-keto-aldonic acids may be produced.

## BIBLIOGRAPHY


1. Bernhard, A., *Science,* **113**, 751 (1951).
2. Bernhauer, K., *Biochem. Z.,* **153**, 517 (1924).
3. Bernhauer, K., *Biochem. Z.,* **172**, 296 (1926).
4. Bernhauer, K., *Biochem. Z.,* **197**, 278 (1928); **197**, 287 (1928).
5. Bernhauer, K., and E. Reidl-Tumova, *Biochem. Z.,* **321**, 26 (1950).
6. Bernhauer, K., and K. Schoen, *Z. physiol. Chem.,* **180**, 232 (1929).
7. Bernhauer, K., and L. Schulhof, U. S. Patent 1,849,053 (1932).
8. Bertrand, G., *Compt. rend.,* **126**, 842, 984 (1898).

8a. Blom, R. H., V. F. Pfeifer, A. J. Moyer, D. H. Traufler, H. F. Conway, C. K. Crocker, R. E. Farison, and D. V. Hannibal, *Ind. Eng. Chem.,* **44**, 435 (1952).

9. Boutroux, L., *Compt. rend.,* **91**, 236 (1880).
10. Boutroux, L., *Compt. rend.,* **104**, 369 (1887).
11. Brown, A. J., *J. Chem. Soc.,* **49**, 172 (1886).
12. Butlin, K. R., "Survey of the Biochemical Activities of the Acetic Acid Bacteria," *Chemical Research, Special Rept. No. 2,* London, H. M. Stationery Office, 1936.
13. Coulthard, C. E., R. Michaelis, W. F. Short, G. Sykes, G. B. Scrimshire, A. F. B. Standfast, J. H. Birkinshaw, and H. Raistrick, *Nature,* **150**, 634 (1942).
14. Coulthard, C. E., R. Michaelis, W. F. Short, G. Sykes, G. B. Scrimshire, A. F. B. Standfast, J. H. Birkinshaw, and H. Raistrick, *Biochem. J.,* **39**, 24 (1945).
15. Currie, J. N., and R. H. Carter, U. S. Patent 1,896,811 (1933).

15a. Currie, J. N., and A. Finlay, U. S. Patent 1,908,225 (1933).

16. Currie, J. N., J. H. Kane and A. Finlay, U. S. Patent 1,893,819 (1933).
17. Foster, J. W., *Chemical Activities of Fungi,* New York, Academic Press, 1949.
18. Franke, W., *Ann.,* **555**, 111 (1944).
19. Franke, W., and M. Deffner, *Ann.,* **541**, 117 (1939).
20. Franke, W., and F. Lorenz, *Ann.,* **532**, 1 (1937).
21. Gastrock, E. A., N. Porges, P. A. Wells, and A. J. Moyer, *Ind. Eng. Chem.,* **30**, 782 (1938).
22. Hermann, S., Austrian Patent 133,139 (1933).
23. Hermann, S., and P. Neuschul, *Biochem. Z.,* **233**, 129 (1931).
24. Herrick, H. T., R. Hellbach, and O. E. May, *Ind. Eng. Chem.,* **27**, 681 (1935).
25. Herrick, H. T., and O. E. May, *J. Biol. Chem.,* **77**, 185 (1928).
26. Hirsch, J., *Istanbul Seririyati,* **25**, No. 8, 20 p. (1943).

27. Isbell, H. S., U. S. Patent 1,976,731 (1934).
28. Isbell, H. S., H. L. Frush, and F. J. Bates, *Ind. Eng. Chem.*, **24**, 375 (1932).
29. Keilin, D., and E. F. Hartree, *Nature*, **157**, 801 (1946).
30. Keilin, D., and E. F. Hartree, *Biochem. J.*, **42**, 221 (1948).
31. Keilin, D., and E. F. Hartree, *Biochem. J.*, **42**, 230 (1948).
32. Lockwood, L. B., U. S. Patent 2,463,784 (1949).
33. Lockwood, L. B., and F. H. Stodola, U. S. Patent 2,496,297 (1950).
34. May, O. E., H. T. Herrick, A. J. Moyer, and R. Hellbach, *Ind. Eng. Chem.*, **21**, 1198 (1929).
35. May, O. E., H. T. Herrick, A. J. Moyer, and P. A. Wells, *Ind. Eng. Chem.*, **26**, 575 (1934).
36. May, O. E., H. T. Herrick, C. Thom, and M. B. Church, *J. Biol. Chem.*, **75**, 417 (1927).
36a. Mehltretter, C. L., M. S. Furry, R. L. Mellies, and J. C. Rankin, *J. Am. Oil Chemists' Soc.*, **29**, 202 (1952).
37. Molliard, M., *Compt. rend.*, **174**, 881 (1922).
38. Molliard, M., *Compt. rend.*, **178**, 41 (1924).
39. Moyer, A. J., U. S. Patent 2,351,500 (1944).
40. Moyer, A. J., O. E. May, and H. T. Herrick, *Centr. Bakt. Parasitenk., Abt. II*, **95**, 311 (1936).
41. Moyer, A. J., E. J. Umberger, and J. J. Stubbs, *Ind. Eng. Chem.*, **32**, 1379 (1940).
42. Moyer, A. J., P. A. Wells, J. J. Stubbs, H. T. Herrick, and O. E. May, *Ind. Eng. Chem.*, **29**, 777 (1937).
43. Müller, D., *Chem. Ztg.*, **50**, 101 (1926).
44. Müller, D., *Biochem. Z.* **199**, 136 (1928).
45. Müller, D., *Biochem. Z.*, **205**, 111 (1929).
46. Müller, D., *Ergeb. Enzymforsch.*, **5**, 259 (1936).
47. Porges, N., T. F. Clark, and S. I. Aronovsky, *Ind. Eng. Chem.*, **33**, 1065 (1941).
48. Porges, N., T. F. Clark, and E. A. Gastrock, *Ind. Eng. Chem.*, **32**, 107 (1940).
48a. Prescott, F. J., J. K. Shaw, J. P. Bilello, and G. O. Cragwell, *Ind. Eng. Chem.*, **45**, 338 (1953).
49. Roberts, E. C., C. K. Cain, R. D. Muir, R. J. Reithel, W. C. Gaby, J. T. Van Bruggen, D. M. Homan, P. A. Katzman, L. R. Jones, and E. A. Dorsy, *J. Biol. Chem.*, **147**, 47 (1943).
50. Schreyer, R., *Biochem. Z.*, **202**, 131 (1928).
50a. Stone, G. B., *Drug and Cosmetic Ind.*, **67**, No. 2, 192 (1950).
51. Takahashi, T., U. S. Patent 1,953,694 (1934).
52. Takahashi, T., and T. Asai, *J. Agr. Chem. Soc., Japan*, **7**, 1 (1931).

53. Takahashi, T., and T. Asai, *J. Agr. Chem. Soc., Japan,* **9**, 351 (1933).
54. Tanaka, K., *Acta Phytochim.,* **7**, 265 (1933).
55. Thies, W., *Centr. Bakt. Parasitenk., Abt. II,* **82**, 321 (1930).
56. Visser't Hooft, F., *Biochemische onderzoekingen over het geslacht Acetobacter,* Thesis, Delft, W. D. Meinema, 1925.
57. Wells, P. A., D. F. J. Lynch, H. T. Herrick, and O. E. May, *Chem. & Met. Engr.,* **44**, 188 (1937).
58. Wells, P. A., A. J. Moyer, J. J. Stubbs, H. T. Herrick, and O. E. May, *Ind. Eng. Chem.,* **29**, 653 (1937).
59. Williams, A. E., *Mfg. Chemist,* **16**, 239 (1945).

CHAPTER 15

# FUMARIC ACID

*Jackson W. Foster*

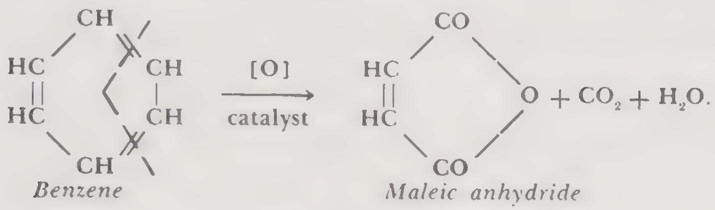
Manufacture of fumaric acid by a microbiological process may be said to be potential rather than actual. Though such a process has been developed and is available, two features have stood in the way of industrial scale application. One is lack of demand for fumaric acid and the other is cost of production. The manufacture of microbiological fumaric acid may be thought of as a counterpart of the kojic acid process; high yields are obtainable, but there is no demand for the product. The cost depends, to a large extent, on potential uses with special reference to possible competing materials.

An important potential use of fumaric acid could be for conversion to maleic acid which, as the anhydride, is consumed in

| | |
|---|---|
| H–C–COOH | H–C–COOH |
| ‖ | ‖ |
| HOOC–C–H | H–C–COOH |
| *Fumaric acid* (*trans*) | *Maleic acid* (*cis*) |

huge quantities in synthetic resins and in paints and varnishes.[6] This conversion is accomplished simply by prolonged heating in acid solution. However, the prevailing belief in the chemical industry today is that the manufacture of maleic acid via fumaric acid is considerably more expensive than the production of maleic

anhydride by vapor-phase oxidation of benzene, using metallic catalysts and atmospheric oxygen:




This is particularly true when the price of fermentation carbohydrate is abnormally high. Should a need for fumaric acid *per se* develop, the microbiological process would stand its best chance for success. Considerable attention is given to the development of a fumaric acid market by one of the two patent assignees of this process, namely Chas. Pfizer and Co., Inc., of Brooklyn, N. Y. This company evidently produces microbiological fumaric on a pilot-plant or small scale to supply the existing relatively minor markets and especially to make available fumaric acid for pilot-plant experimentation on possible new uses. As the cost of fumaric acid diminishes with expanded production, one can expect that it may displace certain other raw materials in manufacturing operations as well as find new uses.

Regarding possible uses, in general, fumaric acid type resins have no advantage over the cheaper maleic anhydride resins. Another serious deterrent to the use of fumaric acid resins is the high quality of phthalic anhydride resins, phthalic anhydride being much cheaper than the $C_4$ dicarboxylic acids.

In some cases, fumaric acid resins have physical and other advantages and thus special applications which make them superior to other resins within the same cost range. This is true of the manufacture of printer's ink. Fumaric acid also yields glycerol-type resins which, used in varnishes, have desirable properties of hardness and durability as compared to the maleic anhydride-glycerol resins commonly used for this purpose. Here again the cost of fumaric acid is the determining factor. Another indicated outlet for fumarate is in the manufacture of wetting agents of the Aerosol type. These are long-chain fatty acid esters of the sodium salt of a sulfonic acid derivative of succinic acid. Maleic anhydride again is the source of the $C_4$ dicarboxylic acid. Should fumarate be cheap enough, it could be used as a maleic substitute.

A possible outlet on a minor scale is based on the fact that magnesium and sodium salts of fumarate have a cathartic action similar to citrates.[21]

## ORGANISMS PRODUCING FUMARIC ACID

Because of its role in intermediary oxidative metabolism first perceived by Szent-Györgyi and formulated in the well-known $C_4$ dicarboxylic acid cycle and later embodied in the Krebs tricarboxylic acid cycle of respiration, fumaric acid is probably produced in all living cells. At least, one may say the two cycles (the first is really a part of the second) are virtually universal in biological systems. Though little work has been done actually to demonstrate this point in fungi, there is no question that these mechanisms operate in some fungi.[9,27] However, the formation of fumaric acid by all cells for performance in intermediary oxidative metabolism is quite a different matter from the formation of fumarate by certain rare organisms as a cumulative end product of metabolism. In the first case, the fumarate is produced in catalytic quantities, is retained within the cells, and is in a dynamic state of equilibrium between malic and succinic acids, being continuously regenerated from these acids. In the rare cases mentioned before, the fumaric acid is excreted from the cells and accumulates in bulk in amounts representing a substantial proportion of the carbohydrate being consumed and no longer participates in metabolism.

So far as is known, fumaric acid production is confined to filamentous fungi. In only a few very specific ones is this ability well developed and these are the ones of potential industrial interest. They belong principally to the order *Mucorales* in the class *Phycomycetes.* Though main attention has been given to the genus *Rhizopus,* specifically *R. nigricans,* fumaric acid production to varying degrees is known to be a property also of several other genera within the order, viz: *Mucor, Cunninghamella* and *Circinella.*[14] Thus, this property seems to be widespread among members of the *Mucorales,* although six different strains of *Absidia,* two of *Phycomyces,* one of *Syncephalastrum* and one of *Mortierella,* tested under the same conditions as the before-mentioned three genera, were negative in this regard. However, the chances are good that this may be a matter of strain specificity since of the genera registered as positive, fumaric acid accumulation was characteristic of only six out of ten strains of *Rhizopus nigricans,* one

of eleven *Mucor* and one of six *Cunninghamella*. Strain specificity is, therefore, just as important in regard to this property as it is in the host of other known physiological processes in microorganisms.

Although large accumulations of fumarate now are known only among the *Mucorales*, it is a fact that fumarate has been isolated in small amounts from numerous other fungus-culture filtrates, generally accompanied by larger amounts of several other more or less related acids. It is not to be doubted that intense microbiological research could improve substantially the yields in these cases, but the effort is scarcely justified considering the high-yielding organisms already known. Examples of organisms which have been reported to produce fumaric acid are: *Penicillium griseo-fulvum*;[24] *Aspergillus glaucus*;[29] *A. flavus, A. oniki*, and *A. wentii*;[30] and *Caldariomyces fumago*.[5] There is little doubt that systematic research would reveal many other fungi which produce from carbohydrates small amounts of fumaric acid in admixture with related acids.

One *Aspergillus*, now only of academic significance, was isolated and described by the eminent German botanist Carl Wehmer[33] some 30 years ago as giving such extraordinarily high yields of fumarate from sugar (up to 70% weight conversion yield) that he named it *Aspergillus fumaricus*. However, Wehmer[34] reported that through continued transfer in the laboratory, stock cultures of this organism underwent physiological degeneration, losing almost entirely its fumaric acid powers and acquiring instead the ability to produce citric and gluconic acids abundantly, similarly to many other Aspergilli. Schreyer[28] made intensive efforts to restore the fumaric acid producing powers of this organism by testing it under a great variety of different nutrient conditions, but was unsuccessful. Shortly afterward, small amounts of fumaric acid were obtained by Thies[31] when the fungus was cultivated submerged by bubbling oxygen through the culture liquid. Though no other *Aspergillus* comparable to *A. fumaricus* has since been described, Wehmer's discovery is proof of their existence in nature. Failure to procure such organisms merely means they have not been systematically sought.

The German biochemist, Felix Ehrlich,[7] discovered in 1911 the high fumaric acid producing potentialities of *Rhizopus nigricans* and the great majority of work since his time has been done with this organism, formerly known as *Mucor stolonifer*. As mentioned

before, not all strains of this species produce fumaric acid, nor are yields comparable. In one instance, strain specificity (i.e., fumaric acid producing ability) was associated with sexuality in these heterothallic fungi, the male or (+) race being a high fumarate yielder, whereas the female or (—) race produced not a trace.[15] Unfortunately the impossibility of germinating the sexual body (zygote) in these organisms and the multinucleate character of the sporangiospores (asexual spores) precludes any attempt to increase the fumaric acid producing powers of this organism by hybridization and other standard genetical approaches.

Interesting physiological differences were revealed between the two highest yielding strains of *Rhizopus nigricans* obtained from 10 tested by Foster and Waksman.[14] These differences were most evident in preformed mycelium replacement cultures. In strain No. 45, practically all of the acidity formed was fumaric acid, but in strain No. 35 substantial amounts of other acids were produced concomitantly, only 55% of the total being accountable as fumaric acid. Preformed mycelium from strain No. 45 produced appreciable amounts of fumarate anaerobically as well as aerobically, but strain No. 35 was active aerobically only. This feature is discussed in detail in the section on mechanisms.

The importance of strain specificity is underscored by the fact that numerous different species of *Rhizopus* produce high yields of lactic acid with none, or only traces, of fumaric acid and others produce varying mixtures of the two. Of sixteen strains of *Rhizopus* tested in three media in surface culture,[25] *R. delemar* gave the highest yields of fumarate (58.8% of glucose consumed), but this was accompanied by 19.4% yield of lactic acid.

## SUBMERGED PRODUCTION OF FUMARIC ACID

This method, as compared to the traditional surface-culture technique, is the only economically feasible microbiological approach to industrial-scale biological production of fumaric acid, yet paradoxically, virtually all the published information on this aspect is limited to the scanty description contained in the two patents covering this process. These were issued in 1943 simultaneously to Waksman[32] and to Kane, Finlay, and Amann.[20] Both patents were assigned each one-half to Merck and Co., Inc., Rahway, N. J. and to Chas. Pfizer and Co., Inc., Brooklyn, N. Y. Submerged production is discussed in several papers.[11,14,22,26]

The Waksman patent stresses the production of fumaric acid in growth cultures and in replacement cultures, whereas the patent of Kane *et al.* stresses only the growth phase. Both patents are very similar. The essence of the process may be represented by citing Example 1 given in the patent of Kane *et al.*:

> "2.7 liters of a 10% invert sugar solution plus 180 grams calcium carbonate (and small amounts of nutrient salts) were sterilized under steam pressure and introduced into a rotary drum-type fermentor. After inoculating with 50 cc. of a selected *Rhizopus nigricans* culture, the drum was started rotating at approximately 15 R.P.M., while being maintained at 33°C and under a pressure of two atmospheres; at the same time, a current of air was kept passing through the fermentor. At the end of four days, the fermentation was stopped and the solution was analyzed, 143 grams of fumaric acid and 24 grams of invert sugar were found."

Thus, 143 g of fumaric acid were produced from 270—24=246 g of invert sugar consumed giving a $143/246 \times 100 = 58\%$ weight conversion yield. Usually, the figure of 64.4% is taken as the theoretical maximum. However, this ceiling figure is based on the assumption that the $C_4$ acid formation takes place exclusively via an aerobic $C_2$—$C_2$ condensation (see later). This is now known to be a fallacy, for another mechanism, independent of oxygen and involving carbon dioxide fixation, has been established to occur in a high-yielding strain of *Rhizopus nigricans.* This means that the theoretical maximum is some substantially higher, but as yet unascertained, value than 64.4%.

The described means of agitation and aeration are not critical and other methods are possible. Instead, on a production scale, the use of high-speed propellers in a stationary tank is the preferred method of agitation, air meanwhile being introduced into the culture liquor in the region under the propeller. For a carbohydrate source, the patents specify a variety of carbohydrates, including glucose, fructose, sucrose, invert sugar, maltose, molasses, and sirups, the various starches, grains, malted grains, cereal products, and other materials containing any of these substances.

Though the cited example does not mention it, the culture filtrate, if it were fully neutralized by the calcium carbonate, would consist of a 7.1% solution of calcium fumarate, almost three times

the solubility of this salt at 30°C. Therefore, by the end of this fermentation, there must have been copious crystallization of calcium fumarate, which, as a matter of fact, probably stopped the process.

With the exception of the two patents, published information dealing with submerged production of fumaric acid is scanty and one is *per force* obliged to extrapolate data available in the considerably more numerous publications dealing with surface cultivation.

## SURFACE PRODUCTION OF FUMARIC ACID

Though of no production significance, a great deal of study has been given to the formation of fumaric acid in surface or stationary cultures of *Rhizopus nigricans* and almost all this information is more or less descriptive of events in submerged culture.

### Medium Constituents

#### Nitrogen Source

Strains of *Rhizopus* are prototrophic with respect to amino acids and growth factors and develop rapidly and abundantly in inorganic nitrogen-salts-glucose media. Like most members of the *Mucorales,* these organisms cannot make use of nitrate nitrogen, but they utilize ammonium nitrogen. Many different forms of organic nitrogen are also suitable, including urea. Doubtless these substances liberate ammonia in which form the nitrogen is assimilated. Ordinarily, ammonium chloride or ammonium sulfate are used as nitrogen sources.

#### Carbohydrate

Simple hexoses and starch can be utilized by species of *Rhizopus* which indeed are among the most powerful diastatic fungi known. Starch has the disadvantage that it gels at 4 to 5% concentration, whereas higher concentrations of carbohydrate are essential for most economical production of fumarate. Sucrose, and thus molasses, is useless for *Rhizopus nigricans* as the fungus lacks invertase. However, simple pretreatment with mineral acid or invertase preparations changes the sucrose to invert sugar, which is utilizable. Inulin-containing substances (levulans) can also be used. Application of crude carbohydrates always results in a much faster growth of the organisms due to organic nitrogenous and inorganic

impurities. The increased growth, in turn, creates aeration difficulties, especially in submerged cultures.

The concentration of carbohydrate essential for maximum fumarate production and conversion yield lies between 5 and 10%, the exact value depending somewhat on other nutritional factors. The maximum weight yield of fumarate does not necessarily coincide with the maximum efficiency of conversion of carbohydrate. Depending on the conditions, it may be expedient to sacrifice efficiency of conversion in the interests of a speedier process of somewhat lower conversion efficiency, this giving the highest economic output per hour from the culture.

In replacement cultures using preformed surface mycelium, the conversion yield of fumarate from glucose is inversely proportional to the initial glucose concentration, as seen in Table 52. Though the largest weight yield of fumarate was obtained with 15% glucose, the conversion efficiency on the basis of glucose consumed was only 30.1% as compared to 46.9% with 2.5% glucose concentration.

TABLE 52. CARBOHYDRATE CONCENTRATION AND FUMARIC ACID CONVERSION EFFICIENCY IN *R. nigricans* REPLACEMENT CULTURES

| Initial glucose concentration % | Glucose consumed g | Fumaric acid formed g | Conversion % |
|---|---|---|---|
| 2.5 | 3.32 | 1.56 | 46.9 |
| 5.0 | 6.75 | 2.65 | 39.3 |
| 10.0 | 10.58 | 3.56 | 33.7 |
| 15.0 | 12.22 | 3.68 | 30.1 |
| 20.0 | 10.06 | 2.99 | 29.7 |
| 25.0 | 10.44 | 1.95 | 18.7 |
| 30.0 | 5.13 | 0.63 | 12.3 |
| 40.0 | 5.13 | 0.40 | 7.8 |

Source: Foster and Waksman.[13]

In reality, the carbon:nitrogen ratio of the medium is the crucial factor. The explanation for this may be found in the theory of shunt metabolism detailed elsewhere by Foster.[9] Using a 5 to 10% hexose solution, the carbon:nitrogen ratio for maximum fumarate yields should range between 50:1 and 100:1. Concentrations of sugar exceeding 10% reduce the rate of sugar consumption.

MINERALS

The usual biological elements must be added, especially if a purified carbohydrate is used. Of greatest significance according to Foster and Waksman[13] is the trace-element nutrition, zinc in particular. It goes without saying that certain trace elements, notably copper, manganese, iron, zinc and possibly a few others must be present in order to obtain any growth of *Rhizopus nigricans,* or any other fungus.[8] When these are not purposely added to the medium, the only reason for occurrence of growth is because they are present as impurities in the medium ingredients, distilled water, glassware, etc., in amounts sufficient to give growth. Different batches of media may vary in their content of impurities and give variable amounts of growth.

Of the trace elements, zinc appears to be quantitatively and qualitatively most important. This is because there is an inverse relation between the amount of cell material synthesized (growth) and fumaric acid produced. In other words, each can be formed in large yields only at the expense of the other. This balance is particularly susceptible to zinc. Thus when the zinc content is limiting, the fumarate yields will be maximum, other factors being suitable. The precise amount of zinc to be added in order to establish the proper balance will, of course, depend on the amount present as an impurity, and will have to be determined under each set of operating conditions; but in general, zinc should be limiting. When crude natural materials are used, such as grain mashes, invert molasses, etc., it is necessary to remove zinc and other growth-promoting substances already present in excess. Table 53 exemplifies the profound influence of zinc on the metabolism of a fumaric acid producing strain of *Rhizopus nigricans.*

TABLE 53. EFFECT OF ZINC ON GROWTH AND FUMARATE PRODUCTION BY *Rhizopus nigricans* PER 200 ML SURFACE CULTURE

| | Glucose consumed g | Fumaric acid produced g | Conversion % | Ammonia nitrogen consumed mg | Total acidity due to fumaric acid % |
|---|---|---|---|---|---|
| Zinc absent | 4.70 | 2.35 | 50.0 | 31.0 | 100 |
| 5 mg $ZnSO_4 \cdot 7H_2O$ added | 5.09 | 0.45 | 8.9 | 82.1 | 71 |

Source: Foster and Waksman.[13]

Growth was two and one-half times greater in the culture containing zinc, using ammonia consumption as a criterion, yet the yield of fumaric acid in this culture was less than one-fifth that of the control containing no zinc. The presence of zinc evidently results in increased synthesis of cell protoplasm with decreased fumaric acid production. Another indication of altered metabolism due to zinc is that when this element was present, appreciable amounts of acids other than fumaric were formed whereas almost no other acidity was present in the zinc-deficient cultures.

Miksch, Rauch, Mielke-Miksch, and Bernhauer[22] obtained, however, increased yields of fumarate by addition of zinc ion. Their data show that the balance between zinc and other trace elements dictates the response to zinc, which may be a positive or a negative one. Thus, 0.6 mg % of $CuSO_4 \cdot 5H_2O$ increased the fumarate yield by 33%, but 1.0 mg % $ZnSO_4 \cdot 7H_2O$, in addition, resulted in a 74% reduction in fumarate. Also, zinc overcame the toxic effect of higher concentrations of copper ion.

## Neutralizing Agents

The very earliest workers with *Rhizopus nigricans* noted that the organism could not tolerate high acidity and that both growth and fumarate formation and accumulation were greatly enhanced by the presence of neutralizing agents. Calcium carbonate has been widely used for this purpose in physiological studies reported in the literature (also calcium hydroxide), but has the disadvantage that the solubility of calcium fumarate is not as high as is desirable for building up the economically feasible highest concentrations of this salt. At 30°C, its solubility is approximately 2.5%. The solubility of the sodium salt exceeds 20%, and Rauch, Miksch, Mielke-Miksch, and Bernhauer[26] report weight conversion yields of 40% of the glucose consumed when sodium carbonate was used as a neutralizing agent to maintain the reaction between pH 5 and 6 (methyl red indicator). The advantage of calcium carbonate is its insolubility, permitting the addition of an excess which neutralizes the fumaric acid as it is formed. Addition of soluble alkalies, like hydroxides of calcium, sodium, or potassium, would have to be done virtually continuously during the fermentation to secure the necessary pH control.

When calcium carbonate is used, it is sterilized separately from the medium and then added aseptically. This prevents generation

of toxic or retarding substances from simple carbohydrates caused by heating in the presence of alkalies.

The crystallization of calcium fumarate in the culture is a serious obstacle to a commercial microbiological process. Near the saturation point, the culture solution becomes very viscous. In surface culture, crystallization begins on the bottom of the vessels and on the lower surface of the mycelial pad. Eventually, the crystallized calcium fumarate becomes so abundant that the entire body of liquid sets in the form of a stiff gel due to the hydrophilic nature of the salt. The situation in submerged cultures was described in the previous section.

## Air Supply

An excess of oxygen is essential for maximum fumaric acid yields. The best demonstration of this feature is that shallow layers of medium give more fumarate in both surface and shake (submerged) cultures. It is also critical in tank (submerged) production. Oxygen deficiency and lowered fumarate yields are always reflected in the accumulation of the other major product of metabolism, a reduced compound: ethyl alcohol.

## Selective Poisons

Miksch and coworkers[22] point the way to higher yields of fumaric acid by artificially distorting the metabolism of *R. delemar* by specific poisons. Thus 0.1% *p*-benzoquinone increased fumarate yield values from 52.2% up to 67.0%, at the expense of ethanol and lactic acid ordinarily accompanying the fumarate. Other poisons, such as selenium salts, sodium fluoride, and iodoacetic acid selectively inhibited fumarate formation and correspondingly induced higher yields of ethanol.

## Reuse of Preformed Mycelium

The reuse of preformed mycelium may be a very important practical aspect of the fumaric acid process, as it has been in physiological studies of the mechanism of formation of fumaric acid. After growth has reached a peak, or the nutrient medium is exhausted, the mycelium is separated from the bulk of the liquid by decantation (in the case of submerged growth, by centrifugation, filtration, or the flotation technique of Porges, Clark and Gastrock[23]). A fresh sterile sugar solution and sterile neutralizing agent

are now introduced into the vessel and incubated in the normal way. Attack on the sugar and fumaric acid production begins at once and at maximum rate. Since only a small part of the substrate is converted to cell substance (oxidative assimilation), maximum conversion yields of fumarate can be obtained. There is no lag phase, nor time lost corresponding to that required for synthesis of cell material in a growing culture.

A single lot of mycelium treated in this way can be used through several consecutive sugar replacements with little reduction in efficiency. Adoption of this technique puts the process on a semicontinuous basis. It works satisfactorily both in surface and submerged cultures.

## MECHANISM OF FORMATION OF FUMARIC ACID

The mechanism of fumaric acid formation has been studied extensively by Butkevich and Fedorov,[1,2,3,4] by Foster and co-workers,[13,14,16,17] and several others. Following the proposal of Gottschalk,[18] most workers in the field assume that classical alcoholic fermentation of the carbohydrate precedes the actual fumaric acid mechanism. This process is anaerobic. The second phase is oxidative, or aerobic. The alcohol is oxidized to acetate, two molecules of which condense via the Thunberg-Wieland condensation to yield one molecule of succinic acid, which, by dehydrogenation, is converted to fumaric acid.

$$\underset{\text{Hexose}}{C_6H_{12}O_6} \xrightarrow[-4H]{} \underset{\text{Pyruvic acid}}{2\,CH_3{\cdot}CO{\cdot}COOH} \xrightarrow[+4H]{\text{decarboxylation}} \underset{\text{Ethyl alcohol}}{2\,CH_3CH_2OH + 2\,CO_2}$$

$$\underset{\text{Ethyl alcohol}}{2\,CH_3CH_2OH} \xrightarrow{+O} \underset{\text{Acetic acid}}{2\,CH_3COOH}$$

$$\underset{\text{Fumaric acid}}{\begin{array}{l} CHCOOH \\ \| \\ CHCOOH \end{array}} \xleftarrow[O]{-2H} \underset{\text{Succinic acid}}{\begin{array}{l} CH_2COOH \\ | \\ CH_2COOH \end{array}} \xleftarrow[O]{-2H} \underset{\text{Acetic acid}}{2\,CH_3COOH}$$

Two major lines of evidence support the genesis of $C_4$ from $C_2$ precursors: (1) Preformed mycelium converts alcohol or acetate into fumaric acid in high yields; (2) alcohol accumulates during the early stages when sugar is being utilized by the fungus and fumarate yields are low. Later, when the sugar is depleted, fumarate formation continues, with a simultaneous drop in the alcohol content.

Attractive as this theory is to interpret fumarate formation

from $C_2$, there still must be eliminated the possibility of the origin of the $C_4$ acid via a complete tricarboxylic acid cycle schematically represented as:

$$C_4 \xrightarrow{+\,C_2} \underset{\textit{cis}\text{-Aconitic acid}}{C_6} \xrightarrow{-\,CO_2} \underset{\alpha\text{-Ketoglutaric acid}}{C_5} \xrightarrow{-\,CO_2} \underset{\text{Fumaric acid}}{C_4}$$

In this case, the fumaric acid results from oxidation of $C_2$ by small catalytic amounts of $C_4$ already present in the cells. However, the formation of each molecule of $C_4$ by this mechanism requires a $C_4$ to begin with; and starting with $C_2$ as the sole substrate the maximum molar yields obtainable would be 66.7%. Yields of fumarate in excess of this figure have been obtained from ethanol by *Rhizopus nigricans*[16] and, therefore, this mechanism cannot be the sole interpretation of fumarate formation in this system.

Experiments with labeled carbon dioxide as a tracer and with nonlabeled ethanol as the substrate show[16,17] that carbon dioxide enters only the carboxyl groups of the fumaric acid (and the small amounts of succinic and malic acids formed simultaneously).

When 2-$C^{14}$-ethanol was used as a substrate, carbon atoms 2 and 3 of the fumaric acid produced arose exclusively from carbon atom 2 of the ethanol. The carboxyls also were labeled. When 1-$C^{14}$-ethanol was used as a substrate, carbon atoms 1 and 4 (carboxyls) of the fumarate were labeled and no labeling was found in carbons 2 and 3. These findings were interpreted as supporting the formation of $C_4$ dicarboxylic acids *via* the Thunberg-Wieland reaction. The fact that carboxyls became labeled when 2-$C^{14}$-ethanol was the substrate was explained in two ways: (1) operation of a respiratory cycle and (2) metabolic exchange between carbon dioxide and carboxyls.

If the Thunberg-Wieland reaction does occur in *Rhizopus nigricans*, then evidence for a $C_4$ dicarboxylic acid cycle of respiration becomes virtually complete, although a $C_6$ tricarboxylic acid may well operate simultaneously to move noncarboxyl labeling to carboxyl positions. Active acetate (2-$C^{14}$ acetate) is assumed to be the condensing form of $C_2$ produced from ethanol. Figure 65 shows how the $C_4$ dicarboxylic acid cycle operates to produce labeled carboxyl groups from labeled methyl groups. It can be seen that after the first cycle, the original methyl-labeled $C_2$ becomes labeled

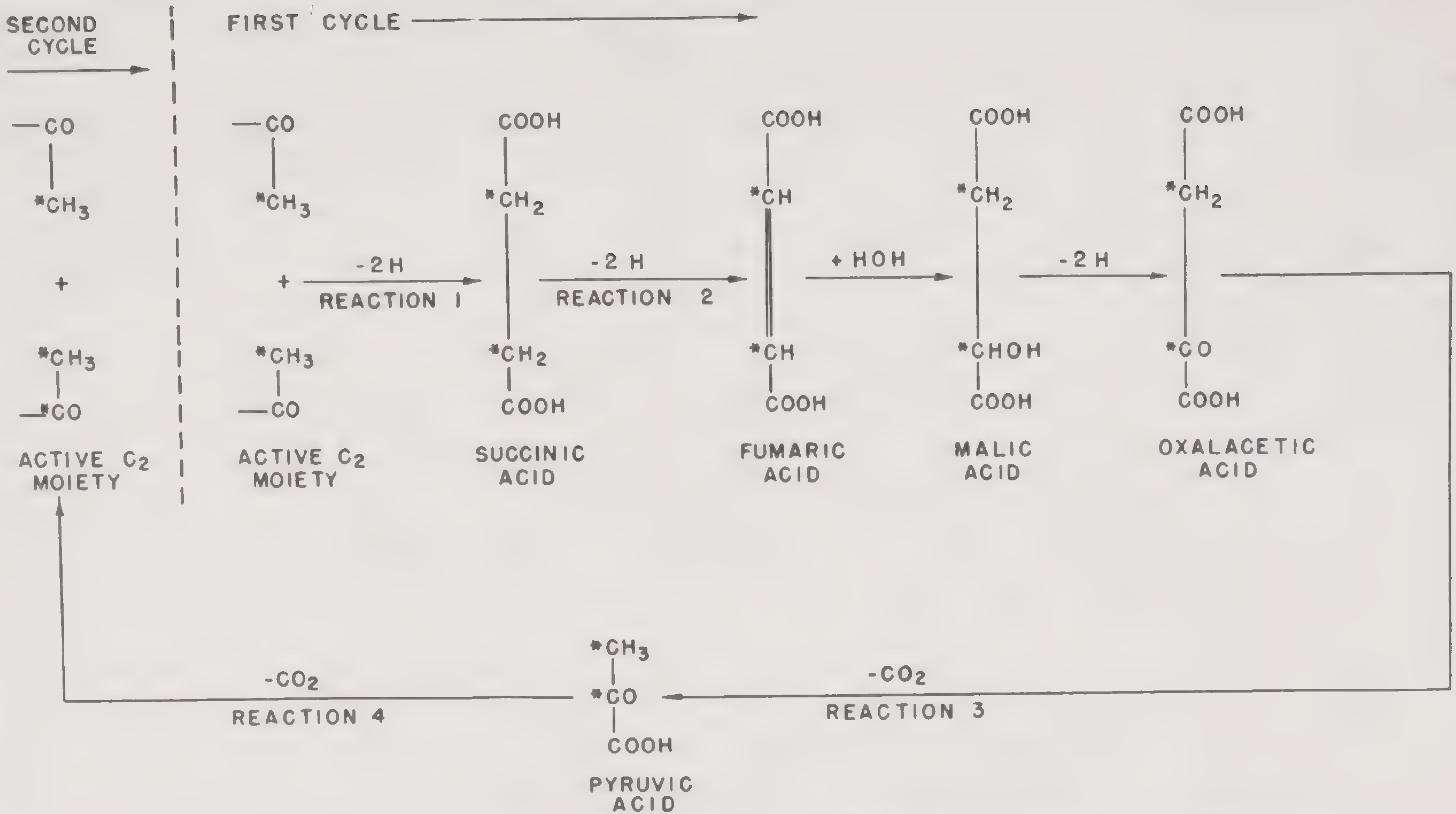



FIGURE 65. *Diagram of* $C_4$ *Dicarboxylic Acid Cycle with Labeled Carbon Compounds*

in both carbons. The resulting participation of the doubly labeled $C_2$ accounts for some of the labeling of carboxyl groups of the $C_4$ dicarboxylic acids.

Labeling of carboxyls of $C_4$ dicarboxylic acids, whose net synthesis is independent of carbon dioxide fixation, occurs *via* the reversible Wood-Werkman decarboxylation of oxalacetate or *via* the reversible Ochoa decarboxylation of malate.

The mechanisms of biosynthesis of $C_4$ dicarboxylic acids is not yet complete, for recent evidence[19] proves that labeled formic acid as a tracer enters all carbons of $C_4$ dicarboxylic acids formed from glucose by *R. nigricans*.

At least one other mechanism for fumarate formation, independent of $C_2$ and independent of oxygen, has been demonstrated. Using a high-yielding strain of *Rhizopus nigricans*, Foster and Davis[11] showed that up to 18% of the carbohydrate consumed, *i.e.*, about one-third of the total fumarate formed aerobically, results from a $C_3 - C_1$ condensation (Wood-Werkman reaction) in the absence of oxygen. Experiments with radioactive carbon dioxide had demonstrated qualitatively[10] the occurrence of this mechanism. The fixed carbon dioxide was shown to reside in the carboxyl group of the fumaric acid. The remainder of the utilized sugar undergoes the usual alcoholic fermentation and some small amount of lactic acid is also formed.

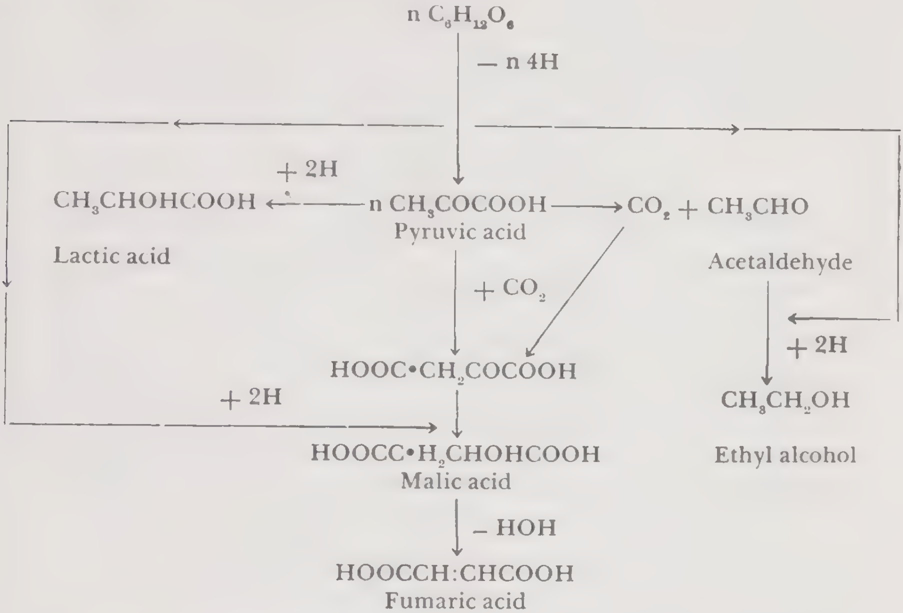
A typical anaerobic fermentation balance for *Rhizopus nigricans* (Strain No. 45) is given in Table 54.

TABLE 54. ANAEROBIC FERMENTATION BALANCE OF *Rhizopus nigricans* NO. 45

| | mg per ml | millimoles |
|---|---|---|
| Glucose consumed | 23.14 | 0.129 |
| Fumaric acid | 3.23 | 0.028 |
| Ethyl alcohol | 8.55 | 0.186 |
| Lactic acid | 4.00 | 0.044 |
| Carbon dioxide | 7.90 | 0.180 |

Source: Foster and Davis.[11]

The following scheme is used to represent the events in anaerobic sugar utilization by this strain of fungus:




In essence, this scheme represents a reduction of pyruvic acid in three ways: before decarboxylation (lactate), after decarboxylation (alcohol), and after carboxylation (fumarate via malate).

Judging from a rather limited number of tests, it appears that not all fumaric acid forming fungi possess the anaerobic mechanism. It appears distinctive to the exceptionally high-yielding strains, which obviously have both mechanisms. Here then is one explanation of strain specificity with regard to fumarate production: Some organisms possess only the aerobic mechanism, and others both mechanisms. High tensions of carbon dioxide specifically inhibit the anaerobic fumarate-forming mechanism at the carbon dioxide fixation reaction.[12] The concomitant alcohol fermentation is only retarded under these conditions.

## RECOVERY OF FUMARIC ACID

The requirement of a neutralizing agent makes the recovery step one of the costliest in the production of fumaric acid. Mineral acid is essential to liberate free acid. On account of its very low solubility in water (0.7% at 25°C), fumaric acid will readily crystallize when a concentrated solution of calcium, sodium, or

potassium salt is acidified. The free acid is easy to recrystallize from hot water. The requirement of large amounts of mineral acid in this step is an important cost factor in this process. The initial liberation of fumaric acid can be done directly on the culture filtrate. To secure a pure, colorless, crystalline product, the crystallization solution is decolorized with activated carbon.

## BIBLIOGRAPHY

1. Butkevich, V. S., and M. V. Fedorov, *Biochem. Z.*, **206**, 440 (1929).
2. Butkevich, V. S., and M. V. Fedorov, *Biochem. Z.*, **207**, 302 (1929).
3. Butkevich, V. S., and M. V. Fedorov, *Biochem. Z.*, **219**, 87 (1930).
4. Butkevich, V. S., and M. V. Fedorov, *Biochem. Z.*, **219**, 103 (1930).
5. Clutterbuck, P. W., S. L. Mukkopadhyay, A. E. Oxford, and H. Raistrick, *Biochem. J.*, **34**, 664 (1940).
6. Doscher, C. K., J. H. Kane, G. O. Cragwell, and W. H. Stalbner, *Ind. Eng. Chem.*, **33**, 315 (1941).
7. Ehrlich, F., *Ber.*, **44**, 3737 (1911).
8. Foster, J. W., *Botan. Rev.*, **5**, 207 (1939).
9. Foster, J. W., *Bact. Rev.*, **11**, 167 (1947).
10. Foster, J. W., S. F. Carson, S. Ruben, and M. D. Kamen, *Proc. Nat. Acad. Sci. U. S.*, **27**, 590 (1941).
11. Foster, J. W., and J. B. Davis, *J. Bact.*, **56**, 329 (1948).
12. Foster, J. W., and J. B. Davis, *Arch. Biochem.*, **21**, 135 (1949).
13. Foster, J. W., and S. A. Waksman, *J. Bact.*, **37**, 599 (1939).
14. Foster, J. W., and S. A. Waksman, *J. Am. Chem. Soc.*, **61**, 127 (1939).
15. Foster, J. W., and S. A. Waksman, *Science*, **89**, 37 (1939).
16. Foster, J. W., S. F. Carson, D. S. Anthony, J. B. Davis, W. E. Jefferson and M. V. Long, *Proc. Nat. Acad. Sci., U. S.*, **35**, 663 (1949).
17. Foster, J. W., and S. F. Carson, *Proc. Nat. Acad. Sci. U. S.*, **36**, 219 (1950).
18. Gottschalk, A., *Z. physiol. Chem.*, **152**, 136 (1926).
19. Jefferson, W. E., D. S. Anthony, E. F. Phares, S. F. Carson, and J. W. Foster, *Proc. Soc. Am. Bact. Ann. Meetings*, 1951.
20. Kane, J. H., A. Finlay, and P. F. Amann, U. S. Patent 2,327,191 (1943).
21. Locke, A., R. B. Locke, H. Schlesinger, and H. Carr, *J. Am. Pharm. Assoc.*, **31**, 12 (1942).

22. Miksch, J. N., J. Rauch, R. Mielke-Miksch, and K. Bernhauer, *Biochem. Z.*, **320**, 398 (1950).
23. Porges, N., T. F. Clark, and E. A. Gastrock, *Ind. Eng. Chem.*, **32**, 107 (1940).
24. Raistrick, H., and P. Simonart, *Biochem. J.*, **27**, 628 (1933).
25. Rauch, J., R. Mielke-Miksch, and K. Bernhauer, *Biochem. Z.*, **320**, 384 (1950).
26. Rauch, J., J. N. Miksch, R. Mielke-Miksch, and K. Bernhauer, *Biochem. Z.*, **320**, 390 (1950).
27. Ryan, F. J., E. L. Tatum, and A. C. Giese, *J. Cellular Comp. Physiol.*, **23**, 83 (1944).
28. Schreyer, R., *Biochem. Z.*, **202**, 131 (1928).
29. Sumiki, Y., *Bull. Agr. Chem. Soc. Japan*, **5**, 10, 13 (1929).
30. Sumiki, Y., *Bull. Agr. Chem. Soc. Japan*, **7**, 62 (1931).
31. Thies, W., *Zentr. Bakt. Parasitenk., Abt. II*, **82**, 321 (1930).
32. Waksman, S. A., U. S. Patent 2,326,986 (1943).
33. Wehmer, C., *Ber.*, **51**, 1663 (1918).
34. Wehmer, C., *Biochem. Z.*, **197**, 418 (1928).

CHAPTER 16

# ITACONIC ACID

*Lewis B. Lockwood*

Itaconic acid has recently been marketed to the chemical industry for experimental purposes. It was produced in pilot-plant-scale operations. Large-scale operation of this fermentation process will depend on economic factors, such as raw materials cost and demand for the product, as well as economy in operation.

While it has been known for many years that itaconic acid is one of the products of the pyrolysis of citric acid, it was only recently discovered to be a metabolic product of certain fungi of the genus *Aspergillus.* Kinoshita[6,7] described a new species, *Aspergillus itaconicus,* which produced itaconic acid and mannitol as the principal products of the metabolism of sucrose. Yields with *A. itaconicus* were too low for industrial use. Calam, Oxford, and Raistrick[2] obtained a 5.9% yield of itaconic acid in 25 days, using a culture of *Aspergillus terreus.* Moyer and Coghill[13] obtained a yield of 27 g of itaconic acid per 100 g glucose supplied to cultures of *Aspergillus terreus.* Eleven mutant strains[8] obtained by irradiation of Moyer and Coghill's culture with ultraviolet light produced slightly greater yields. However, still higher-yielding strains were isolated from natural sources and are now used in experimental and pilot plant studies. Yuill[20] recently reported the simultaneous production of itaconic and kojic acids by a yellow *Aspergillus.*

Yields of itaconic acid as great as 20% of the sucrose supplied were found in cultures which contained calcium carbonate. The calcium salts of both itaconic and kojic acids crystallized under the pellicles. A patent has been issued to Kane, Finlay, and Amann,[5] covering the production of itaconic acid from molasses or invert sugar, in agitated aerated media, in the presence of sodium alginate. Yields as great as 27.5% are claimed.

## RAW MATERIALS

Glucose was the raw material used in most of the published studies on the production of itaconic acid by *A. terreus.* The acid is also produced when sucrose is fermented. Both the glucose and fructose moieties of the sucrose molecule are converted equally well to itaconic acid. Yields of itaconic acid obtained from glucose and sucrose are equivalent on a hexose-unit basis. Accordingly, molasses should be the cheapest raw material for this fermentation.

## MECHANISM OF THE FERMENTATION REACTION

The mechanism of the itaconic acid fermentation is unknown. The structural and chemical relationships of itaconic, aconitic, and citric acids suggest that one of these compounds may be a precursor of the other, or that both itaconic and citric acids may be derived from a common precursor.

| | | |
|---|---|---|
| $H_2C-COOH$ | $H_2C-COOH$ | $H_2C-COOH$ |
| \| | \| | \| |
| $HOC-COOH$ | $C-COOH$ | $C-COOH$ |
| \| | \|\| | \|\| |
| $H_2C-COOH$ | $HC-COOH$ | $CH_2$ |
| Citric acid | Aconitic acid | Itaconic acid |

The close relationship of the mechanisms for the production of itaconic and citric acids is also suggested by the marked similarity of conditions under which these acids accumulate in culture media. The optimal pH of the culture solutions for the production of each of these acids has been reported to be 1.8. This pH value is too low for the *Aspergilli* to make their greatest yield of mycelium.

## CULTURES AND CULTURE MAINTENANCE

*Aspergillus terreus* is widespread in the warmer soils of the world. There are great differences in the ability of isolates to produce itaconic acid, so that selection of suitable cultures is very

important. The mold grows well on either semisolid or liquid media. On Czapek agar medium, it produces a white surface mycelium, but does not produce a good supply of spores. On crude vegetable-extract media, or media which contain malt extract, wort, or corn steep liquor in addition to glucose, abundant sporulation occurs rapidly. The malt-agar slant cultures can maintain viability in storage for 6 months to 1 year in a refrigerator at 4° to 6°C. The spores can be put up in sterile soil according to the method of Greene and Fred,[4] or they can be suspended in blood serum and preserved by instantaneous freezing followed by desiccation from the frozen state. Itaconic acid yields of lyophilized cultures stored by this method are as good as those obtained under the same conditions prior to storage, or from cultures which have undergone frequent transfer.

## DEVELOPMENT OF INOCULA

In mold fermentations, the preparation of inocula constitutes a separate phase of the process, quite different from the actual fermentation. Usually sporulation is undesirable in the final fermentation culture, but is necessary to the preparation of a good inoculum. Consequently, the conditions for the development of inocula are markedly different from those maintained in the fermentation cultures. A good inoculum is characterized by the presence of an adequate number of spores on a thin, fragile mycelium. In many organisms, sporulation results when conditions become unfavorable for vegetative growth, due either to starvation or toxic conditions. If the starvation is due to an inadequate supply of nutrients, both mycelial growth and sporulation may be restricted. Physiological starvation on account of high osmotic pressure, in the presence of an adequate supply of nutrients, frequently results in the production of an abundant supply of spores on the mycelia. A composition of medium which meets the requirements for sporulation and mycelial growth of some strains of *Aspergillus terreus* is as follows: glucose monohydrate 22 g, ferric tartrate 0.005 g, $MgSO_4 \cdot 7H_2O$ 0.1 g, $KH_2PO_4$ 0.2 g, $NaNO_3$ 1.2 g, NaCl 35 g, water to make 1,000 ml.

## THE FERMENTATION

Itaconic acid may be produced by either the surface- or submerged-culture methods. The submerged-culture method should

be the easier process to operate, but slightly greater yields have been reported for the surface process than for the submerged fermentation. Various factors affecting production of itaconic acid by *A. terreus* have been discussed by Lockwood and Reeves,[10] Lockwood and Ward,[9] Lockwood and Nelson,[11] Lockwood and Moyer,[12] Nelson, Traufler, Kelley, and Lockwood,[14] and Pfeifer, Vojnovich and Heger.[14a]

In the surface fermentation process, the solution is fermented in shallow pans, the depth of the solution being 1.5 to 2 in. One square centimeter of sporulation culture should inoculate 250 sq cm of surface culture. When the depth is 1.5 in., 1 sq cm of inoculum mat should inoculate 1 l of solution. If germinated spores are used to inoculate shallow, unagitated cultures, mycelium formation on the surface of the solution will take a day or two longer than is required when dry spores are spread evenly over the surface.

A suitable nutrient composition for the shallow-pan process contains per 1,000 ml: glucose monohydrate 275 g, $MgSO_4 \cdot 7H_2O$ 4.5 g, $NH_4NO_3$ 2.5 g, NaCl 0.4 g, $ZnSO_4 \cdot 7H_2O$ 0.0044 g, $HNO_3$ (sp. gr. 1.42) 1.6 ml, and corn steep liquor 4 ml. Air, saturated with moisture at 24°C, is blown over the surface of the culture at the rate of approximately 1 l per minute per 1,000 sq cm of surface. The fermentation is complete in 12 days. The pH of the solution is approximately 2.2 during the fermentation and should not rise above 2.3, since at higher pH values, itaconic acid is metabolized readily by surface growing pellicles of *A. terreus.*

Germinated spores are suitable for the inoculation of shaker cultures in which the mycelium is grown submerged in the solution. Such inocula may be employed in the form of small discrete spherical pellets, or may be dispersed uniformly throughout the medium. A large inoculum results in excessive mycelial growth and little itaconic acid production. Use of much smaller inocula results in lesser mycelial growth and larger itaconic acid yields. Using pellet-type inocula that have been allowed 2 days for germination and early growth, eight to sixteen pellets per liter of culture solution gives the best acid yields.

A nutrient composition satisfactory for the production of itaconic acid in shaker cultures should contain per 1,000 ml: glucose monohydrate 66 g, $MgSO_4 \cdot 7H_2O$ 0.75 g, $NH_4NO_3$ 2.5 g, ferric tartrate 0.25 g, corn steep liquor 4 ml, $HNO_3$ (sp. gr. 1.42) 1.6 ml.

Sulfuric acid may be substituted for the nitric acid. It is necessary to maintain the pH at 1.8 by frequent addition of acid or ammonia as required during the fermentation. The fermentation is completed in about 8 days.

This fermentation process has never been operated on a scale larger than pilot plant. If itaconic acid is manufactured by the surface-culture method it is possible to use either stainless-steel or aluminum pans. Enough aluminum will dissolve in the mash at pH 2.2 to prevent growth of *A. terreus,* unless the magnesium content of the mash is about ten times as high as that ordinarily used in culture media. The high magnesium content of the medium is advantageous also in that yields of itaconic acid on such media are considerably greater than in media of lower magnesium content. In surface cultures, an inadequate air supply results in excessive mycelial growth and reduced yield of acid. A considerable excess of air is permissible over the 1 l per 1,000 sq cm per minute recommended previously.

It is evident, from the nature of the process as described, that for submerged culturing, the fermentors must be constructed of an acid-resistant metal. Certain stainless steels, for example 334SS, are suitable for fermentor construction. If much corrosion occurs, the presence of excessive iron in the mash will result in reduction in itaconic acid yield and in reduced life of the equipment.

Nelson, Traufler, Kelley, and Lockwood[14] conducted 15-l *A. terreus* fermentations in 20-l stainless-steel fermentors and described optimal conditions somewhat different from those reported for shake-flask culture.[11] The initial glucose concentration was 6% in corn steep liquor medium. The pH during the fermentation was maintained at 1.8 to 1.9. Other conditions considered optimum were: (1) the use of sulfuric acid rather than nitric acid for pH adjustments; (2) 1% inoculum; (3) a constant supply of available nitrogen, 2.67 g $(NH_4)_2SO_4$ per l being satisfactory; (4) the use of 5.0 g $MgSO_4 \cdot 7H_2O$ per l; and (5) aeration at the rate of one-thirtieth volume of air per volume of medium per minute.

Pilot-plant investigations of the itaconic acid fermentation were reported recently by Pfeifer, Vojnovich, and Heger.[14a] Stock spore cultures of the organism *A. terreus* NRRL 1960 were carried on medium containing 2.5% malt extract, 0.1% peptone, 2% glucose, and 2.0 g agar. Variables influencing the formation of itaconic acid were investigated in 300- and 600-gal fermentors of stainless steel

over a range of conditions. These factors included methods of sterilization, antifoam agents, medium composition, inoculum, fermentation pressure, aeration and agitation, fermentation temperature, and pH. From the experimental results, the following procedures were recommended for the successful fermentation of glucose to itaconic acid.

The medium for both inoculum development and for itaconic acid production should contain 6.60% glucose monohydrate, 0.27% $(NH_4)_2SO_4$, 0.08% $MgSO_4 \cdot 7H_2O$ and 0.18% corn steep liquor. Media may be sterilized continuously at 300°F for 5 minutes, or in batches at 250°F for 30 minutes, the pH in batch sterilization being reduced to 5.0 with itaconic acid to prevent loss of ammonia. For seed development, 1% by volume of inoculum suffices, but 5 to 10% of inoculum should be used in the production fermentors. Fermentation temperature of 95°F, aeration at the rate of one-eighth volume of air per minute per volume of medium, pressure of 10 to 20 psi gage, and moderate agitation are suitable, with 0.75% octadecanol in 95% ethanol added as antifoam agent as required. Maximum yields of about 60 g of itaconic acid per 100 g anhydrous glucose supplied are obtained after 48 to 72 hours of fermentation.

Vicenty and coworkers have investigated the production of itaconic acid from sucrose by *A. terreus* both in surface[18] and in submerged[19] fermentations. In surface culture,[18] the optimum conditions were a fermentation period of 12 days on a 1.7 cm deep medium, a pH of 1.8 to 2.3, and a sucrose content of 14 to 17%. Yields from fermentations of brown cane sugar were 41%. Boiled or decanted sugar-cane juice was also satisfactory as a substrate. From technical glucose, itaconic acid yields of 36 to 38% were obtained by surface culture.

The yield of itaconic acid from sucrose by submerged culture of *A. terreus* was found to decrease with increased concentration of the sugar.[19] Using a 20-l fermentor with mechanical agitation and aeration at the rate of 15 l of air per minute, with the sucrose medium at pH 1.8 to 1.9, a yield of 38% itaconic acid was obtained in 7 to 8 days.

## CONTAMINATION PROBLEMS

Since the pH of the fermentation solution in surface cultures must be held at 1.8, there is no danger of contamination by bacteria and little danger of contamination by fungi other than members of

the *A. niger* and *A. wentii* groups. Itaconic acid yields of cultures contaminated with molds of these types may be very poor. In the pilot-plant submerged-culture experiments of Pfeifer, *et al.*,[14a] the fermentations were started at an initial pH of 5.0 and contamination was prevented by pure-culture technique throughout the process. In two of more than eighty pilot-plant fermentations, contamination by *A. niger* occurred and in each case, acid production stopped abruptly.

## ANALYTICAL METHODS

Only about 85% of the acid produced in this fermentation is itaconic, the remainder being principally succinic and itatartaric acids, or itatartaric lactone. Since both of these acids are saturated, while itaconic acid is unsaturated and brominates readily, Friedkin[3] developed a bromination method for the determination of itaconic acid in culture liquors. The reagent contains 1 ml bromine, 3.0 g potassium bromide, 1.87 g potassium chloride, 48.5 ml 1 $N$ hydrochloric acid, and water to make 500 ml. The bromine and potassium bromide are dissolved in a small amount of water before the other ingredients are added. The reagent has a pH of 1.2, which is too low for the bromine oxidation of glucose to interfere in the determination.

To a 1 or 2 ml sample in a 125-ml iodine flask, 50 ml of this bromine reagent is added. The stopper is sealed with water. After 10 minutes at room temperature and 5 minutes in an ice bath, 5 ml of potassium iodide solution (50 g potassium iodide in 100 ml of water) is poured around the stopper. The stopper is carefully lifted, so that the solution is sucked into the flask by the vacuum created by cooling in the ice bath. After standing for 10 minutes, the iodine liberated is titrated with 0.1 $N$ thiosulfate, using starch as indicator. The titer of 50 ml of the bromide reagent, treated in the same way as the sample, is used as a blank value. The difference in milliliters between the blank titer and the titer obtained with a sample is equivalent to the milliliters of 0.1 $N$ itaconic acid in the sample.

## YIELDS AND RECOVERY METHODS

In the surface-fermentation process, yields of itaconic acid as great as 38 g per 100 g of sugar supplied have been obtained when 15% glucose solutions were fermented in shallow pans. In sub-

merged culture, the yields have been 30 to 33% when 6% glucose was fermented in small, agitated cultures, in excess of 50% in a 20-l fermentor,[14] and up to 65% in pilot-plant fermentations.[14a]

The acid is recovered by evaporation of the liquor to less than one-tenth its volume after the mold mat from surface cultures has been removed, washed, and expressed, or the mycelium has been filtered from submerged cultures. The acid crystallizes in the form of a slurry which is readily separated by filtration or centrifugation. A second crop of crude itaconic acid may be obtained from the mother liquor on concentration to half its volume. This second crop of crystals is best recycled with filtered beer to be concentrated. In this manner about 90% of the total acid crystallizes and, after washing the crystals with cold water and drying, a product of 96 to 98% purity is obtained. It is light brown in color and is suitable for esterification for use in resin synthesis without further treatment. Further purification to a white product is accomplished by dissolving the tan crystals in hot water to make a 25% solution, decolorizing hot with 2% carbon, filtering, crystallizing, centrifuging, and drying. This treatment results in 80% recovery of white crystals of 99+% purity; 1% is lost with the decolorizing carbon; and the mother liquor is recycled in the recovery process. Other recovery processes have been suggested, such as extraction with *n*-butyl or amyl alcohol, from which it crystallizes on evaporation of the solvent. The resulting product may be somewhat contaminated with succinic and itatartaric acids[17] and gummy residues from the residual sugars or neutral nonreducing compounds, such as erythritol[16] formed during the fermentation. There is a possibility that the crude itaconic acid could be purified on a commercial scale by sublimation.

No data are available on waste disposal or by-product recovery. The wastes consist of washed mycelia and concentrated fermented liquor from which the acids have been removed. It might be possible, but would probably not be economical, to recover the succinic and itatartaric acids. The recovery of erythritol and other neutral nonreducing compounds, representing 20 to 30% of the original sugar, would depend on the development of markets for them.

## ECONOMICS

Cost figures for the manufacture of itaconic acid by fermentation are not available. However, Pfeifer *et al.*[14a] have estimated the

plant production costs at 28¢ per pound for 97% tan itaconic acid, or 31¢ per pound for 99+% white acid, with corn sugar at 7.5¢ per pound. These plant production costs do not include administrative and selling expenses. It appears probable that considerable reduction in cost would be possible if molasses could be substituted for glucose, since the sugar content of molasses costs about one-third as much as that of glucose and items other than sugar add relatively little to the raw-material cost. However, in the several attempts made by Pfeifer *et al.*[14a] to replace refined glucose with cane or beet molasses or hydrol, little itaconic acid was produced. Further investigation of changes in medium composition and operating conditions, or in pretreatment of the molasses, might make possible the utilization of these cheaper raw materials.

## USES AND COMPETITIVE PROCESSES

The principal uses for itaconic acid appear in the resin and detergent industries.[1,14a] Itaconic acid is, chemically, a substituted methacrylic acid, and its esters polymerize to produce products similar to those of the methacrylate series. For such use, itaconic acid esters must compete with methacrylate esters in price and availability. The price of high-quality lactic acid, or of acrylonitrile, is a factor in this price competition. Itaconic acid forms polyester resins with dihydric alcohols, which, when blended with styrene, may be used for low-pressure adhesives. Oil-modified alkyd resins derived from itaconic acid may have some use as coating materials. In the detergent field, itaconic acid will have to compete with maleic, fumaric, and other dicarboxylic acids for a place in the manufacture of Aerosol-type wetting agents.

A competitive chemical process for the manufacture of itaconic acid involves the conversion of calcium aconitate to itaconic acid by heating in acid solution.[15] Calcium aconitate is obtained as a by-product of the recovery of sugar from cane molasses. The pH of the liquor from the second crop of sugar crystals is adjusted with calcium hydroxide and the precipitated calcium-magnesium salts, principally calcium aconitate, are allowed to settle out. It has been estimated that improvement in the third crop of sugar crystals is sufficient to merit the extra operations involved in the process and that calcium aconitate can be marketed for 15 cents per pound. On acidification and heating, aconitic acid decarboxylates, producing principally itaconic acid.

An additional method for producing itaconic acid, the pyrolysis of citric acid, probably will offer little competition with the fermentation process, unless considerable improvement is accomplished in this method.

## BIBLIOGRAPHY


1. Anon., *Chemical Industries,* **60**, 946 (1947).
2. Calam, C. J., A. E. Oxford and H. Raistrick, *Biochem. J.,* **33**, 1488 (1939).
3. Friedkin, M., *Ind. Eng. Chem., Anal. Ed.,* **17**, 637 (1945).
4. Greene, H. C., and E. B. Fred, *Ind. Eng. Chem.,* **26**, 1297 (1934).
5. Kane, J. H., A. C. Finlay, and P. F. Amann, U. S. Patent 2,385,283 (1945).
6. Kinoshita, K., *Acta Phytochim.,* **5**, 271 (1931).
7. Kinoshita, K., *Acta Phytochim.,* **9**, 129 (1937).
8. Lockwood, L. B., K. B. Raper, A. J. Moyer, and R. D. Coghill, *Am. J. Bot.,* **32**, 214 (1945).
9. Lockwood, L. B., and G. E. Ward, *Ind. Eng. Chem.,* **37**, 405 (1945).
10. Lockwood, L. B., and M. D. Reeves, *Arch. Biochem.,* **6**, 455 (1945).
11. Lockwood, L. B., and G. E. N. Nelson, *Arch. Biochem.,* **10**, 365 (1946).
12. Lockwood, L. B., and A. J. Moyer, U. S. Patent 2,462,981 (1949).
13. Moyer, A. J., and R. D. Coghill, *Arch. Biochem.,* **7**, 167 (1945).
14. Nelson, G. E. N., D. H. Traufler, S. E. Kelley, and L. B. Lockwood, *Ind. Eng. Chem.,* **44**, 1166 (1952).

14a. Pfeifer, V. F., C. Vojnovich, and E. N. Heger, *Ind. Eng. Chem.,* **44**, 2975 (1952).

15. Roberts, E. J., J. A. Ambler, and A. L. Curl, U. S. Patent 2,448,831 (1948).
16. Stodola, F. H., *J. Biol. Chem.,* **166**, 79 (1946).
17. Stodola, F. H., M. Friedkin, A. J. Moyer, and R. D. Coghill, *J. Biol. Chem.,* **161**, 739 (1945).
18. Vicenty, C., P. M. Paradis, L. E. Cancel, D. Alcala, and R. Fernandez-Garcia, *El Crisol (Puerto Rico),* **4**, 51 (1950).
19. Vicenty, C., P. M. Paradis, L. E. Cancel, D. Alcala, and R. Fernandez-Garcia, *Bol. Colegio. quím. Puerto Rico,* **7**, 26 (1950).
20. Yuill, J. L., *Nature,* **161**, 397 (1948).

CHAPTER 17

# ACETIC ACID–VINEGAR

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Vinegar has been known and appreciated as an important food adjunct (condiment and preservative) for as long as man has been able to practice the arts of brewing and wine making. It is mentioned in the early classical literature, the Bible, was known by and occupied an important place among the compounds of the alchemists, and formerly was a popular nostrum.

Vinegar, derived from the French *vinaigre,* means literally sour or sharp wine (vin = wine; aigre = sour or sharp). It may be defined as the product resulting from acetification of alcoholic solutions derived from sugary or starchy raw materials. The word "vinegar" has lost its original meaning in the United States, for here, unless used with a qualifying adjective, it denotes only the acetified product made from fermented apple juice.

Vinegar may be produced from a wide variety of raw materials, the main requirements being a satisfactory, economic source of alcohol and accessory flavoring constituents. Apple or cider vinegar is the common table vinegar used in the United States. Wine vinegar is used extensively in the large grape-growing regions of Europe. Malt vinegar is very popular in England. Distilled vinegar, also known as spirit, grain or white vinegar, is commonly used in

the food industries, although certain specialty products owe their distinctiveness to wine or cider vinegar. Although apples, grapes, grain and molasses are the chief raw materials used for the production of vinegar, it may also be produced from a variety of surplus products. Such commodities as fresh pears, peaches, plums, figs, oranges, pineapples, and berries can be used for the production of vinegar.[9]

Distinctive condiment vinegars may be produced from dried fruits, including prunes, apples, figs, apricots, peaches, and dates. Honey vinegar also is distinctive.[12] Hydrolyzed starchy substances, such as potatoes, rice, corn, wheat, and other grains may also be used. Sugar vinegar is produced from sugar sirup, molasses, or refiner's sirup. Glucose vinegar is made from glucose solutions. It is to be stressed that whatever is used as the raw material, it must first undergo alcoholic fermentation before acetification can proceed on an economic basis.

Two basic processes for the production of vinegar are known. The "slow" process, also known as the Orleans or French method, was used extensively for a long time before the "generator" process was developed. The generator process now developed to a reasonably high state of efficiency first came into prominence a century ago, largely through the efforts of Schüzenbach, who introduced the method into Germany.

The practical conditions necessary for the manufacture of vinegar from alcoholic solutions were known in an empirical way a long time before the oxidation could be explained on a scientific basis. Many of the scientific discoveries credited to others in all probability were technical secrets of the vinegar manufacturers long before the scientific discoveries were recorded in the literature. In any event, many of the advances in our knowledge of vinegar production cannot be credited to one individual. For example, it is probable that the living nature of "mother of vinegar" was suspected before it was actually suggested by Boerhaave,[5] a century before Persoon[28] made the first botanical study of "mother of vinegar" and Kützing[20] recognized that the minute organisms of the "mother" were responsible for acetification of alcohol.

Most of the other early developments also apparently resulted from the additive efforts of several individuals. Boerhaave has been generally credited with the first vinegar generator, yet Mitchell[25] cites an anonymous description of a rudimentary generator pub-

lished in 1670. The absolute necessity for air in the process of making vinegar also must have been recognized before Rozier[31] observed the absorption of air during the acetification and Lavoisier[21] demonstrated that the oxygen of the air was the essential ingredient.

Despite the ancient, natural origin of vinegar, acetous beer and wine, its manufacture has changed little in the past century. The nature of the process, however, has been substantially elucidated.

## COMPOSITION OF VINEGAR

As already stated, two entirely different microbial processes are required for the production of vinegar. The raw material used must first undergo an alcoholic fermentation. This alcoholic solution, known as "vinegar stock," then is acetified by oxidation.

### Composition of Vinegar Stock

The hexose sugars of the raw material, whether there at the beginning or formed by enzymic or chemical hydrolysis, are converted by species of yeasts of the genus *Saccharomyces* into alcohol through anaerobic fermentation. This biochemical conversion may be represented by the simple reaction,

$$\underset{\text{Hexose}}{C_6H_{12}O_6} \xrightarrow{\text{Yeast enzymes}} \underset{\text{Alcohol}}{2\,CH_3CH_2OH} + \underset{\text{Carbon dioxide}}{2\,CO_2}.$$

This reaction is only an approximation. It does not take into consideration that some residual hexose is left unfermented or that small amounts of other products are formed in the fermentation. Thus, the solution to be acetified may contain, in addition to alcohol, traces of sugar (hexose), glycerol, formic, acetic, lactic, and succinic acids, acetylmethylcarbinol, and other constituents characteristic for the raw material used. These natural ingredients include malic and tartaric acids, esters, pigments, pentosans, proteins, and minerals.

Abnormal vinegar stock may contain unusual quantities of certain of these compounds because of microbial decomposition of the ingredients of the stock or the raw material. Lactic acid bacteria (species of *Lactobacillus* and *Leuconostoc*) may cause significant increases in acetic and lactic acids and impart a "mousey" taste by decomposition of the sugar contained in the raw material or the

resultant vinegar stock. Species of the same bacteria may decompose malic acid with the formation of lactic acid or cause the formation of mannitol from fructose if either malic acid or fructose is present in the raw material or "stock." Abnormal increases in methyl alcohol may result from the enzymic decomposition of pectin caused by molds in raw fruits, particularly apples and peaches.

## Composition of Vinegar

The alcohol contained in the vinegar stock is converted to acetic acid by oxidative enzymes produced by the acetic acid bacteria. This second mandatory stage in the production of vinegar may be represented by the reaction,

$$2\,CH_3CH_2OH + 2\,O_2 \xrightarrow{\text{Acetobacter enzyme system}} 2\,CH_3COOH + 2\,H_2O$$

In reality, however, the acetification of alcohol is a step-wise oxidation. Acetaldehyde, the chief intermediate, must be formed before acetification can occur. The oxidation of alcohol to acetic acid, therefore, is more correctly represented when written as two reactions,

$$CH_3CH_2OH + O \xrightarrow{\text{Acetobacter enzyme system}} CH_3CHO + H_2O$$
$$CH_3CHO + O \xrightarrow{\text{Acetobacter enzyme system}} CH_3COOH$$

The acetification of vinegar stock never goes to completion, so that alcohol, together with other minor constituents, and products of fermentation of the raw material are present in the finished product. These constituents include, among others, aldehydes, esters, acetylmethylcarbinol, glycerol, and lactic, malic and tartaric acids, the last being found only in wine vinegar.

## Definitions and Standards

The following definitions and standards have been used by the Food and Drug Administration of the United States Department of Agriculture for purposes of regulation since 1936:[36]

*Vinegar, cider vinegar, apple vinegar.* The product made by the alcoholic and subsequent acetous fermentations of the juice of apples. It contains in 100 ml (20°C) not less than 4 g of acetic acid.

*Wine vinegar, grape vinegar.* The product made by the alcoholic and subsequent acetous fermentations of the juice of

grapes. It contains in 100 ml (20°C) not less than 4 g of acetic acid.

*Malt vinegar.* The product made by the alcoholic and subsequent acetous fermentations, without distillation, of an infusion of barley malt or cereals whose starch has been converted by malt. It contains in 100 ml (20°C) not less than 4 g of acetic acid.

*Sugar vinegar.* The product made by the alcoholic and subsequent acetous fermentations of sugar sirup, molasses and refiner's sirup. It contains in 100 ml (20°C) not less than 4 g of acetic acid.

*Glucose vinegar.* The product made by the alcoholic and subsequent acetous fermentations of a solution of glucose. It is dextrorotatory and contains in 100 ml (20°C) not less than 4 g of acetic acid.

*Spirit vinegar, distilled vinegar, grain vinegar.* The product made by the acetous fermentation of dilute distilled alcohol. It contains in 100 ml (20°C) not less than 4 g of acetic acid.

Seasoned vinegars, prepared by infusion of cider or wine vinegar with herbs, spices, eschallots or garlic, frequently are preferred for condiment purposes. These vinegars must be labeled to indicate the seasoning used and the source of the basic vinegar. The minimum amount of acetic acid allowed for such vinegars is also 4 g in 100 ml (20°C).

Formerly, the definitions for vinegar described in detail the chemical characteristics of the solids. The "standard" finally included the requirement that not more than half of the apple solids should be reducing sugars. It apparently was not realized that the best vinegar stock contained very little residual sugar and very frequently also contained less than the required 1.6% of total apple solids. As a result, the producer of quality vinegar would have been compelled to adulterate his product if he expected it to meet the requirements of the "standard."[35]

Certain practices are permissible if they are not deleterious to the public health and if the product is correspondingly labeled. For example, vinegars may be diluted to not less than standard strength, but the dilution must be plainly declared on the label. It makes no difference when the water was added; the label must carry the declaration that it was added. Label declaration of mixing of vinegars made from different raw materials is also required. These mixtures are labeled "compound." Vinegars which have been treated with harmless flavors or colors to imitate a particular

type may be sold if the label carries the statement, "imitation vinegar."

The question of compliance with the definition is the obligation of the analyst who may make his decision after studying all data pertaining to a sample either on the basis of his own experience or by comparison with known samples.

The detection of artificial or adulterated vinegars sometimes is a complicated task. The best approach is to determine the presence or absence of substances known to be normal ingredients of the various types of vinegar. Acetylmethylcarbinol is one of the characteristic compounds found in nearly all vinegars of biological origin. Formic acid, however, is sometimes found in appreciable quantity in vinegar compounded from synthetic acetic acid, but is present in only very small amounts in natural vinegars.

Analyses may include such determinations as specific gravity, solids, ash, solubility and alkalinity of ash, alcohol, total acidity, nonvolatile acids, volatile acid, polarization, total reducing substances before and after inversion, sugars, volatile reducing substances, acetylmethylcarbinol, soluble and insoluble phosphoric acid, permanganate oxidation value, mineral acids, pentosans, formic acid, and glycerol.

With a fairly detailed analysis and a thorough knowledge of the analytical variations of the natural vinegars, it is possible to determine if the sample is genuine or, if adulterated, the probable adulterants.

## PRODUCTION DATA

Completely satisfactory data to illustrate the annual total production of vinegar in the United States are not available. Similarly, it is not possible to estimate accurately the annual production of cider, malt, wine, distilled, and miscellaneous vinegars.

In 1939, the production of vinegar amounted to 107,040,462 gal with a value in excess of $11,000,000 (U. S. Dept. of Commerce data). Statistics shown in Table 55 indicate that the annual production of vinegar packed in glass averages about 6,250,000 cases per year. These figures do not take into account the quantities made on farms, in small vinegar plants, and as a by-product of canning and other establishments not primarily engaged in the manufacture of vinegar. Substantial quantities of vinegar are produced and used in cucumber pickles, catsup, salad dressings,

and in other food products, without the vinegar having left the premises as such.

TABLE 55. UNITED STATES PRODUCTION OF VINEGAR PACKED IN GLASS

| Year | Cases[a] |
|---|---|
| 1941 | 6,250,000 |
| 1942 | 6,250,000 |
| 1943 | 6,000,000 |
| 1944 | 6,990,000 |
| 1945 | 5,900,000 |
| 1946 | 6,500,000 |
| 1947 | 6,000,000 |

[a] Standard case holds four one-gallon jugs.

Source: *Western Canner and Packer,* Statistical review and yearbook number, **40**, (No. 5), 233, (1948).

## METHODS OF PRODUCTION

Despite a popular fallacy which has persisted for about 40 years, the known species of *Acetobacter* do not convert significant quantities of glucose and other carbohydrates directly into acetic acid. Vinegar raw material must undergo an alcoholic fermentation.

### Preparation of Vinegar Stock

Preparation of the vinegar stock depends on the nature of the raw material. If starchy products (grains or potatoes) are used, the starch must be converted by enzymic or acid hydrolysis to obtain a readily fermentable source of hexose sugar. Dried fruits must be infused with water in order to obtain the fruit sugars in solution. Fresh fruits, such as apples or grapes, must be crushed and the juice expressed by pressure. Other raw materials containing large amounts of sugar, such as molasses or honey, are diluted with water. Whatever the raw material, it must contain at least 8% of sugar, and preferably more, as prepared for the alcoholic fermentation, if the finished vinegar is to contain the legal minimum acetic acid content of 4 g per 100 ml.

Control of the alcoholic fermentation is desirable to insure an economic conversion of sugar to alcohol and to obtain a better-quality vinegar stock, as reflected by its pleasing flavor and aroma. The lactic acid bacteria dissipate the sugar with production of lactic and acetic acids and also impart a "mousey" off-flavor and odor,

particularly to fruit juices. If acetic acid is produced in any quantity, the fermentation may cease before all of the sugar has been converted. A concentration of 0.5% acetic acid markedly diminishes the activity of the yeast and higher concentrations will completely inhibit the alcoholic fermentation. In the absence of alcohol which they can oxidize, the acetic acid bacteria may also dissipate sugar with the production of gluconic or ketogluconic acid, or polysaccharides and cause development of the same "mousey" off-flavor as produced by the lactic acid bacteria. The film-forming oxidative yeasts must also be controlled, for they also consume sugar as well as alcohol.

The yeasts naturally present in the fruit juice or other raw material may cause a satisfactory fermentation. However, in many cases, a spontaneous fermentation may be very undesirable because of the resultant competition between the yeasts and bacteria. To insure a rapid, clean, efficient alcoholic fermentation, the raw material should be inoculated with a selected yeast starter.

Fermentations are often started by the addition of brewer's or baker's yeast *(Saccharomyces cereviseae).* Grape-juice fermentations are usually started with a selected strain of wine yeast *(Saccharomyces cereviseae* var. *ellipsoideus).* Starters are used at the rate of 2 to 10% by volume and, once the fermentors become heavily seeded with yeast, the starters may be discontinued.

Sulfur dioxide is sometimes used to control the undesirable bacteria during the alcoholic fermentation of apple and grape juices. If it is used, care must be taken to eliminate all but traces of free $SO_2$ in the vinegar stock before acetification, because it is very toxic to the acetic acid bacteria. Storage of the stock for long periods, or mixing with untreated stock, will generally permit binding of the free $SO_2$. The free $SO_2$ may also be eliminated by vigorous aeration or, more easily and quickly, by addition of chemicals to cause binding or oxidation. If used, sulfur dioxide should be used sparingly; 100 ppm should give desirable protection if used properly.

During the primary stage of fermentation which normally lasts from 3 to 7 days, the bulk of the sugar should be converted to alcohol. The remaining sugar is slowly converted during the secondary fermentation which extends over a period of several weeks or more. Because the secondary fermentation is slow, the fermenting solution should be transferred from the open fermentor to a closed one where it can be kept under anaerobic conditions to protect it

from attack by aerobic yeasts and the acetic acid bacteria as well as from undue chemical oxidation.

When the fermentation is complete, it is necessary to remove the yeast cells and other debris by sedimentation. A storage period of several weeks is allowed for settling, after which the supernatant stock is drawn off (racked). The stock may then be stored for further stabilization, racking, and aging, or it may be acetified directly.

If the vinegar stock is to be stored, it is almost mandatory to place it under anaerobic conditions. If stored in the open, the stock is subject to serious loss of alcohol by evaporation as well as by metabolism by oxidative yeasts. The unprotected stock is also subject to increased chemical oxidation which causes undesirable color changes. Furthermore, storage of vinegar stock in open tanks is subject to very severe criticism from the standpoint of sanitation. Vinegar flies and other insects would be attracted in great numbers. Some most certainly would contaminate the stock. It also might be contaminated by rodents and birds if left unprotected.

Care must be also taken to protect the vinegar stock against the attack of lactic acid bacteria, if the stock is to be stored. Probably the best way to accomplish biological control is to denature the stock with vinegar. Addition of enough vinegar to give 1 g of acetic acid per 100 ml is sufficient to control the lactic acid bacteria. As has already been mentioned, sulfur dioxide may be used for the same purpose.

Chemical and physical control of the fermentation generally is very simple, particularly in those plants which produce only cider-vinegar stock. The course of the fermentation may be followed with Balling or Brix hydrometers which indicate the approximate amount of sugar present. When the fermentation is complete, the amount of alcohol by volume may be determined with the ebulliometer. Only in exceptional cases are other chemical determinations made on the bulk of the cider stock. Furthermore, even though it is considered desirable by most, control of the temperature of fermentation is not regularly practiced in the preparation of cider-vinegar stock. However, rigid chemical and physical (temperature) control is customary in the fermentation of the majority of the wines, grain and other raw materials which eventually may be used for the production of vinegar.

## Blending of Vinegar Stock

Apple juice is the only raw material commonly fermented by the vinegar producers; wine and alcohol for the vinegar stock is purchased from the winery and distillery. Apple juice, although it contains an average of 10.5% and a maximum of 15.4%, may contain as little as 6.5% sugars as invert, according to Chatfield and McLaughlin.[8] If the juice contains 8% or less sugar, it should be blended with other juices to increase the sugar content to a satisfactory level.

Calculation of the stoichiometric yields shows that 1 g of hexose (glucose) will give 0.511 g of alcohol or 0.667 g of acetic acid, and 1 g of alcohol will yield 1.304 g of acetic acid. According to some of the literature, the over-all conversion of sugar is in the range of 0.50 to 0.55 g of acetic acid from 1 g of sugar, equivalent to an over-all conversion of 75 and 82.5% of stoichiometric theoretical, respectively. Commercial yields, unfortunately, are considerably less than those indicated in the literature or obtained in the laboratory. Whereas the industrial conversion of sugar to alcohol may be as high as 85 to 90% of stoichiometric theoretical, the oxidation of alcohol to acetic acid is never so efficient.

In practice, the "rule of thumb" assumption has been that 1 ml of absolute alcohol would yield 1 g of acetic acid by oxidation in the generator. This has given rise to the assumption that 1 g of absolute alcohol yields 1.26 g of acetic acid by biological oxidation. Records examined by the author show that the efficiency is much lower than the $1.26/1.304 \times 100 = 96.5\%$ efficiency this would indicate.

In the industry, a conversion averaging 1 g of acetic acid from 1 g of alcohol is considered economical, a yield of $1.0/1.304 \times 100 = 76.6\%$ of theoretical. A continuous yield of 1.1 g of acetic acid from 1 g of alcohol is considered exceptional, a yield of $1.1/1.304 \times 100 = 84.4\%$ of theoretical. Therefore, if the conversion of sugar to alcohol was 90% of theoretical and the subsequent oxidation of the alcohol to acetic acid was 85% efficient, then a raw material containing 8 g of sugar would yield $8 \times 0.667 \times 0.90 \times 0.85 = 4.08$ g of acetic acid. Since, however, the conversion of sugar to alcohol generally averages about 85% efficiency, the raw material would yield $8 \times 0.667 \times 0.85 \times 0.85 = 3.84$ g of actic acid, if the oxidation of alcohol to acetic acid remained at 85% efficiency.

It is, therefore, quite obvious that the efficiency of conversion must be very rigidly controlled to insure that the finished vinegar will contain enough acetic acid to always comply with the legal minimum requirements if the raw material contains only 8% hexose sugar.

## ACETIFICATION

Vinegar may be made either by slow acetification of vinegar stock in barrels or by rapid oxidation in generators. Formerly, much vinegar was made as a home industry simply by allowing wine or cider to oxidize spontaneously in containers open to the air. Now, however, most commercial vinegar is produced in generators, although until the period of World War II some commercial quantities of wine vinegar were still produced by the slow barrel process.

### Slow Acetification

The slow acetification process commonly used for production of commercial quantities of vinegar is known as the French or Orleans process. This method is the oldest and is conceded to be the best process for the production of the finest quality table vinegars.

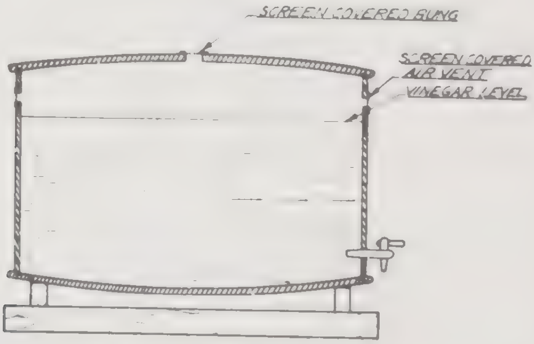



FIGURE 66. *Orleans Process Barrel for Making Wine Vinegar*

Oak barrels of 50 to 54 gal capacity have been used in California as the containers in the process.[19] To start the process, barrels fitted with air ports, as shown in Figure 66, are filled one-fourth to one-third full with good, sound vinegar, containing an active population of acetic acid bacteria. Vinegar stock is then added in quantities to fill the barrel to one-half to two-thirds of its

capacity. The mixture is allowed to remain in the barrel until it has reached its maximum acetic acid content. A portion equal to two-thirds to three-fourths of the vinegar is withdrawn and replaced with an equal quantity of fresh vinegar stock and the acetification repeated.

The process can be made more or less continuous by modification of the routine of filling and withdrawal. The barrels are filled about one-third full of a mixture of fresh, unpasteurized vinegar and 3 or 4 gal of stock. Then, at weekly intervals for 1 month, more vinegar stock is added. After 5 weeks the desired volume of vinegar is withdrawn and replaced with fresh stock. By regulation of the amount of new stock added to insure its acetification in 7 days, the process then becomes, in a sense, continuous, as vinegar can be withdrawn at weekly intervals.

Because the process is slow, taking from 1 to 3 months, or even longer, according to the temperature, the vinegar thus produced contains a larger quantity of esters (principally ethyl acetate) than does vinegar made in the generator from the same lot of stock. The method is particularly adapted to the production of the finest quality wine vinegar. However, the method, by present standards, is too slow and uneconomical of labor and raw materials to be used other than for production of small quantities of wine vinegar for the most discriminating consumers.

Various modifications of the slow process have been devised.[3,25] All of these improvements were intended to obtain the supposedly desirable aerobic film of acetic acid bacteria and to increase the oxidizing surface in order to increase the rate of acetification.

## Rapid Acetification

Rapid acetification of vinegar is a process variously known as the generator process, quick vinegar (Schnellessig), the German process, the Boerhave or the Schüzenbach process. The generator process was introduced into Germany in 1832 by Schüzenbach although, as already indicated, essentially the same method, described in 1670, was in use in France. The generator process came into prominence during the past century. It is now in almost universal use for the production of most commercial vinegars.

The vinegar generator is designed to provide the maximum surface exposure for a volume of vinegar stock in order to supply enough air for the acetic acid bacteria to efficiently and quickly

oxidize the alcohol to acetic acid. Oxidation of the alcohol is accomplished by droplet dispersion of the vinegar stock which is applied to the upper surface of a mass of packing medium (beechwood shavings, coke, etc.) of some depth. The vinegar stock trickles through the generator packing. The acetic acid bacteria present on the generator packing find conditions satisfactory for rapid oxidation of the alcohol. The acetification taking place simultaneously and rapidly throughout most of the exposed surface of the generator medium explains why the term "quick vinegar" is commonly used to denote the generator process.

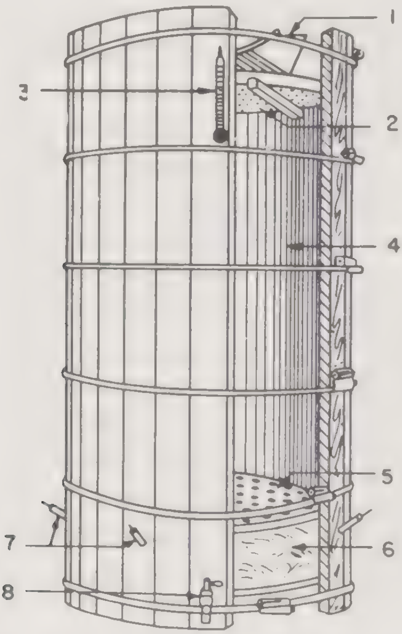



FIGURE 67. *Simple Vinegar Generator*

The generator consists of a large, cylindrical, straight-sided to slightly tapered (conical) wooden tank divided into three compartments (a simple form is shown in Figure 67). Oregon fir, Louisiana cypress and California redwood are commonly used in construction. The upper compartment contains the distribution apparatus to insure even application of the vinegar stock over the generator medium. The central compartment may be filled with beechwood shavings, coke, wood charcoal, basketwork made of rattan, bundled rattan, excelsior, corncobs, grape steams, grape

pomace, ceramic materials or other materials which offer large surface areas. It is essential that the packing material shall not impart undesirable flavors or odors to the vinegar or contain metals (iron and copper) in appreciable quantities. The generator packing must be thoroughly extracted with water and then with vinegar before use. The lower chamber serves as a sump for the collection of the vinegar and contains ports for the admission of air.

In operation, the vinegar stock is distributed intermittently over the generator medium in small but constant amounts. The intermittent application results from the use of automatic tipping troughs or revolving spargers. If the dosage of vinegar stock is continuous, the liquid tends to channel and there is a consequent loss of efficiency from overloading certain parts of the generating medium.

The oxygen necessary for the bacteria is supplied by the air which enters the generator through the ports or vents in the sides of the generator in the bottom compartment (Figure 67). The air passes up through the packing and out through the loose-fitting top, its passage upward being assured by the heat generated by acetification of the vinegar stock.

The temperature of the generator must be carefully regulated. In the simple generator (Figure 67), the temperature is regulated by adjustment of the rate of flow of the vinegar stock down through the generator and the volume of fresh air passing up through the packing to balance the temperature at 80° to 85°F. In the large, closed, recirculating-type generator (Figure 68), the air may be pumped through the generator at a more or less constant rate and the temperature controlled by cooling the vinegar stock. In the United States and in Germany, the generators are commonly operated at temperatures of 80°F to not over 90°F. In England, however, according to Mitchell, generator temperatures may rise as high as 105° to 110°F for optimum operation.

The necessity for rigid control of both air supply and temperature is best illustrated by consideration of stoichiometric relationships involved in oxidation and the heat of oxidation of alcohol to acetic acid. The amount of air required and heat generated from the oxidation of alcohol to acetic acid can be illustrated with type calculations starting with 1 gal of distilled vinegar stock containing 11% alcohol by volume, equivalent to 8.83% alcohol by weight. Therefore, 1 gal of stock contains $0.0883 \times 3785 \times 0.985 = 329.11$

g of alcohol. To convert the alcohol to acetic acid $329.11 \times 64/92 = 228.93$ g of oxygen are required. This represents $288.93/32 = 7.15$ moles of oxygen, (first equation, page 501) which are equivalent to $7.15 \times 22.4 = 160.16$ l of oxygen. Since air is approximately one-fifth oxygen, $160.16 \times 5 = 800.8$ l of air or $800.8 \div 0.0353 = 28.27$ cu ft of air are required to oxidize the alcohol in the 1 gal of stock. In reality, the cubic feet of air required should be at least twice this volume because the oxygen of the air apparently is not fixed quantitatively by the bacteria in the generator. With lower concentrations of alcohol in the stock, the amount of air required is, of course, less.

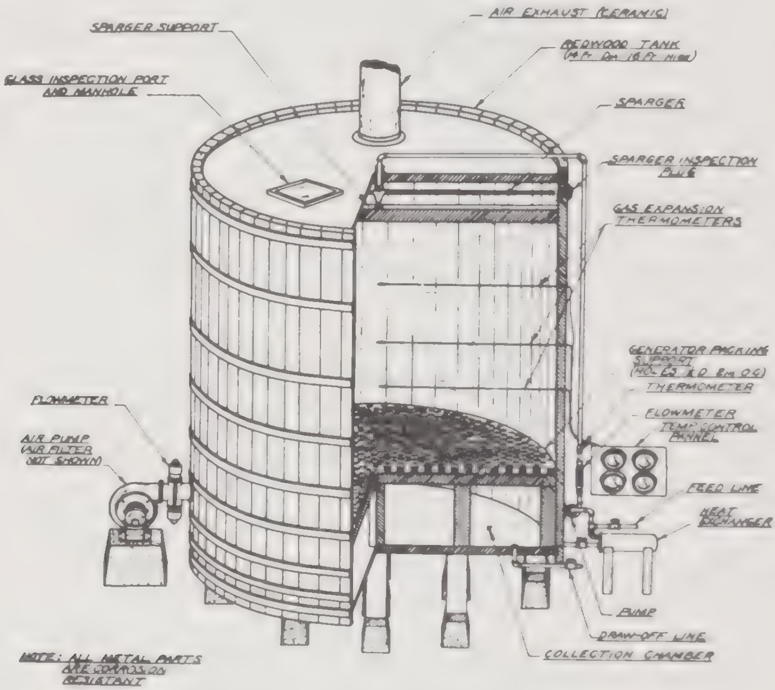



FIGURE 68. *Recirculating-Type Vinegar Generator*

According to Cruess,[10] 1 mole of alcohol will liberate 115 Cal of heat during its conversion to acetic acid. This corresponds to $115/46 = 2.5$ Cal per gram of alcohol. The gallon of vinegar stock contained 329.11 g of alcohol. Therefore, it can liberate $329.11 \times 2.5 = 822.78$ Cal or $822.78 \times 3.9685 = 3{,}265.2$ Btu in its conversion to vinegar. In the conversion of vinegar stock in the large generators, where as much as 2,000 to 2,500 gal may be oxidized, it is obvious that the control of heat would be a com-

plicated problem if none was lost by radiation. In any event, care should be taken to keep the temperature of the generator between 80° and 90°F. At 98° to 105°F, some acetic acid bacteria may be completely inactivated.

Much alcohol and acetic acid are lost in the generator process through evaporation. In the "slow" Orleans process, evaporation losses may be as high as 10% according to Bioletti.[4] A small amount of alcohol (about 0.5% by volume) is always left in the finished vinegar, since it is uneconomical to oxidize all of it. Small or large amounts of alcohol and acetic acid may be lost by complete oxidation (combustion) to carbon dioxide and water. Some may be utilized for the growth of the vinegar bacteria and some may be dissipated in side reactions. These losses, while never eliminated, may be reduced by proper attention to regulation of temperature and air supply in the generator.

## Generator Production Capacity

According to data published by the Hydraulic Press Manufacturing Co.,[18] for every bushel of shavings in the generator, the yield should be 0.25 gal of 6% cider vinegar in 24 hours. The bushel is equivalent to 1.24 cu ft of shavings so the production capacity of 1 cu ft of shavings is $0.25 \times 1/1.24 = 0.20$ gal of 6% vinegar in 24 hours. This may be considered a minimum, since the production rates may go as high as 0.50 gal of 6% vinegar in 24 hours. It is impossible to predict how the generator will work before it is actually placed in operation. Therefore, it is common practice to figure the production capacity of the generator on the basis of 1 cu ft of packing material producing 0.2 to 0.3 gal of 6% vinegar in 24 hours.

Generators vary widely in over-all size, the smallest in commercial operation being about 4 to 5 ft in diameter by 8 ft tall while the largest may be from 14 to 18 ft in diameter by 16 to 20 ft tall.

## Seeding the Generator

As already mentioned, the packing material in the generator should be washed, preferably with hot water, and then soaked in vinegar to insure the removal of off-tastes and odors. Then, before the generator can be placed in operation, it is necessary to seed (inoculate) the packing with acetic acid bacteria. This is generally

accomplished by the use of fresh, sound, unpasteurized, unclarified vinegar taken from a generator of proved high production efficiency. The fresh vinegar is slowly recirculated through the generator for 8 to 12 hours. On the second day, enough alcohol is added to the vinegar to bring it to 2 to 3% by volume and the mixture is again slowly recirculated for 8 to 12 hours. The generator is then shut down under reduced air draft until the bacteria have multiplied on the surfaces of the packing medium to such an extent that the temperature of the interior of the generator starts to increase. Once this happens, the generator is ready for operation.

An alternative procedure is to use fresh, unpasteurized, incompletely oxidized vinegar containing 2 to 3% of alcohol by volume. This is very slowly recirculated through the generator under reduced air draft until the temperature increase indicates that the generator is seeded.

Inoculation of the generator will take 7 to 10 days on the average. Under exceptional circumstances, seeding may be accomplished in less time. It is easy to seed the cider or wine vinegar generator in comparison with the distilled vinegar generator. Distilled vinegar is essentially a synthetic mixture and contains no natural nutritive substances (growth factors) as do cider and wine. It is, therefore, necessary to add inorganic and organic nitrogenous substances to distilled vinegar if it is being used for seeding purposes.

It is to be stressed that the use of pure cultures of acetic acid bacteria for the production of vinegar is impractical and uneconomical, although it might be inferred from the literature that they should be used. The generators, storage tanks, and other wooden equipment could not be sterilized and operated under aseptic conditions. Pure-culture techniques and equipment that would be required to produce the starters of desirable acetic acid bacteria would be uneconomical.

Heavily seeded starters of a desirable species of *Acetobacter* have been used to control the bacterial populations in the Orleans process for wine vinegar. None of the stock was pasteurized before inoculation. This technique might, under certain conditions, be applicable to generators. The method does not insure pure cultures: only a dominant population whose period of domination in the generator is unknown.

Vinegar is too inexpensive to support costly and complicated controls required in the use of pure cultures.

**Operation of the Generator**

Once the generator is seeded, its operation will depend on whether it is designed to be used as a "one-run" generator, to be operated in tandem with one or more others, or as a recirculating generator.

The "one-run" process, as the name implies, provides for one passage of a mixture of vinegar stock and vinegar through the generator. In this type of operation, the original stock is acidified with enough vinegar to increase the acidity to between 3 and 3.5%. Acetification in the generator increases the acidity of the finished vinegar to about 6%.

In the tandem process, the vinegar stock is passed through one generator to become partially acetified. Acetification is completed by passage of this partially oxidized stock through a second or even a third generator.

In the recirculating type of generator, the stock is continuously repumped through the packing until the alcohol is oxidized. When the stock is prepared for oxidation in generators operated in tandem or in the recirculating generator, it may be acidified with vinegar to about 1% acetic acid or used without acidification.

The idea of recirculating vinegar stock through the generator is at least 125 years old. Wüstenfeld[45] cites a patent issued to Ham in England in 1824. Recirculation also apparently was practiced in the U. S., according to Bioletti,[4] for some time prior to its popularization by the work of Frings.[14] The recirculating generator has become increasingly popular because it has several economic advantages. It may be operated at a low cost. It is relatively simple and easy to control. There is a distinct saving of space and equipment. The recirculating generator needs less cubic feet of packing to produce the same quantity of vinegar in the same time. The temperature of the outside air does not affect the generator adversely, since the vinegar stock is used to cool the generator, thus providing for a steadv, efficient production of vinegar unaffected by seasonal variations in temperature. There is no accumulation of fumes of alcohol, acetaldehyde, and acetic acid, since the generator is airtight and stacked to the outside atmosphere. The absence of fumes is a distinct advantage from the standpoint of sanitation, because vinegar flies are not attracted to the same extent.

Cider and wine vinegar, as well as distilled vinegar, are pro-

duced in the recirculating generator. In fact, cider and wine vinegar are more easily produced in this type of generator than is distilled vinegar. However, control of sliming, caused by the growth of *Acetobacter xylinum,* is a serious problem in the production of cider and wine vinegar in the recirculating generator, or any type of generator for that matter, although it never interferes in the production of distilled vinegar.

The nutritional requirements of *Acetobacter xylinum* are well fulfilled with either cider- or wine-vinegar stock. The bacterium requires complex organic nitrogen and alcohol or other carbohydrate material to initiate and maintain growth. To produce the thick, slimy, cellulosic membrane, hexose sugar or other carbohydrates found in cider- or wine-vinegar stock is needed. Distilled vinegar stock normally contains too little of the required nutrients and too much alcohol for the growth of *Acetobacter xylinum.* Thus it is obvious why *Acetobacter xylinum* will, unless controlled, sooner or later produce enough slime to partially or even tightly plug the cider or wine vinegar generator but not the distilled vinegar generator.

In practice, the economic period of operation of the cider or wine vinegar generator will average about 6 months unless extreme care is taken to prevent entrance of *Acetobacter xylinum.* When care is exercised the cider or wine vinegar generator may continue to operate effectively for a year before the slime causes severe reduction in efficiency. Slime formation can be minimized by using wine-vinegar stock with enough alcohol to produce vinegar containing 10% acetic acid. However, because this bacterium is so widely distributed in the industry, most producers, rather than attempt to keep it out of the generator, use packing materials which are almost indestructible or so inexpensive that no significant loss occurs when the packing is washed and cleaned. Then, when the generator becomes so plugged as to be uneconomical to operate, the packing is removed from the shell and thoroughly cleaned. Coke, rattan bundles, and corncobs are the packing materials commonly used in place of beechwood shavings for generation of cider and wine vinegar.

Because the distilled vinegar generator does not become plugged, it is generally filled with beechwood shavings (Figure 69). Distilled-vinegar generators will operate for an unknown period of years without becoming plugged with *Acetobacter xylinum* mem-

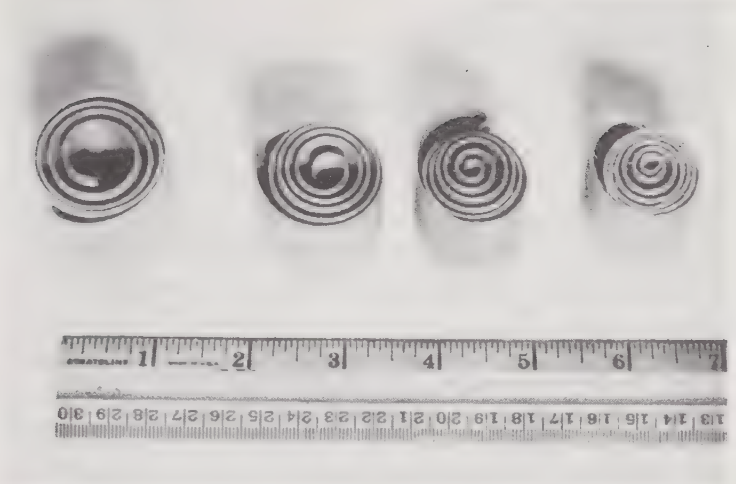



FIGURE 69. *Beechwood Shavings. At the extreme left, the shaving is too losely curled; at the extreme right, the shaving is too tightly curled*

brane. However, the distilled vinegar producer has another bacteriological problem which is troublesome. As already mentioned, distilled-vinegar stock is almost devoid of nutrients. The alcohol used is required by law (consult Regulations 3, 7 and 19 of the United States Treasury Department, Bureau of Internal Revenue for the legal requirements[37,38,39,40]) to be denatured either with ethyl acetate or distilled vinegar, unless the alcohol has been produced by the vaporizing process. The denatured alcohol is suitably diluted with water to prepare the vinegar stock. To oxidize the resultant stock, it is necessary to add nutrients which will support the growth and oxidative activity of the acetic acid bacteria. Various mixtures of inorganic and organic nitrogenous compounds are used. These include various combinations of dibasic ammonium phosphate, urea, asparagine, peptones, yeast autolysate, etc. Several compounded "bacteria foods" are available and many prefer to use them rather than develop their own formulas. The bacterial nutrients generally are added to the distilled vinegar stock in concentrations of 1 to 5 lb per 1,000 gal.

Frequently, a distilled vinegar generator operates at a reduced capacity because the bacteria apparently do not function properly. Sometimes, the efficiency of the generator may be increased by

adding more nutrients or by adding different nutrients. However, in some cases, no increase in oxidative capacity is forthcoming, even when supposedly optimum physical and chemical conditions are maintained for the bacteria. When this occurs, it is advisable to reexamine the generator, giving careful attention to all factors which might affect the activity of the bacteria. If the generator design and construction are found to be satisfactory, then it is desirable to attempt to increase the efficiency of the generator by reseeding it with a new population of bacteria.

Allgeier, Wisthoff, and Hildebrandt[1a,1b] have recently undertaken a series of experimental studies of the factors affecting the performance of distilled-vinegar generators. They state[1b] that studies of such variables as composition, degree of chlorination and biological purity of dilution water, nutrients employed, factors leading to improved aroma, and various other elements involved in vinegar manufacture might be expected to raise production levels and improve the quality of the vinegar. They employ small-scale recirculating generators described by Hildebrandt.[16a]

The first paper of Allgeier, Wisthoff, and Hildebrandt[1a] reported a study of the efficiency of acetification resulting from the use of six dilution waters from widely separated parts of the United States. Considerable difference in the suitability of the various dilution waters for generator operation was found, but difficulty was experienced in correlating the generator performances with the chemical analyses of the waters. Generally speaking, the better waters were those with low solids, hardness, and chloride content. In the second paper[1b] these workers reported that the quality of finished vinegar, as judged by aroma, was best from alcohol denatured with ethyl acetate, second from alcohol denatured with vinegar, and poorest from alcohol denatured with acetaldehyde. It was also reported that the approximate toxicity threshold for the presence of five metals tested in the vinegar stocks was lead 10, copper 15, iron 50, zinc 100, and tin greater than 100 ppm. In all cases toxic amounts of the metals resulted in a decrease in efficiency of acetification in the generators.

## Other Processes

Several interesting modifications of the two basic methods of vinegar manufacture have been made. One of the first attempts to

speed up acetification by the Orleans process was the shallow-vat method of Pasteur.[27] In this method, large, shallow, covered troughs with holes drilled in the sides above the level of the liquid markedly increased the surface of the wine-vinegar stock exposed to the air. Various modifications of the shallow-vat method have been described.

There are also many varieties of the generator process. These include the dipping generator, the displacement generator, the revolving-drum generator, and the large-room generator.

The dipping generator consists of an upright tank inside of which a wooden or wicker basket filled with beechwood shavings can be raised or lowered. The generator tank is filled about one-half full of vinegar stock and the basket of shavings is lowered into the stock. After the requisite time the basket is raised out of contact with the stock in the tank and a large air vent, in the middle of the side of the tank, is opened. The basket of shavings, saturated with the stock, is left exposed to the air for time enough to oxidize the alcohol. The process is then repeated.

The displacement generator consists of a tank fitted with an inner floating tank and two generators at the side of the main tank. When the floating tank is filled with water or some other heavy material, vinegar stock contained in the main tank is forced by displacement into the two generators. When the packing in these generators is saturated, the displacement tank is emptied, thus permitting the flooded generators to drain back into the main tank.

The horizontal revolving-drum generator consists of an inner cylinder so constructed as to admit a maximum amount of air to the packing material contained in it. The generating drum is enclosed in an air-tight outer cylinder or tank fitted with air vents. Half of the generator drum is exposed to the air and half submerged in the vinegar stock as the drum is rotated.

The large-room generator (Grossraumbildner) is a large, rectangular, closed, recirculating generator. It was designed by Frings in Germany. This generator and other interesting modifications are described in detail by Wüstenfeld,[45] Mitchell,[25] and Verona.[43] Since none of them are of commercial importance, they will not be discussed here.

The most recent modifications of the modern generator process consist chiefly in improvements in design and instrumentation. All

such improvements are intended to increase the efficiency and ease of operation. (See Fetzer,[13] Frings,[14] Hansen,[15] Speas,[33] Elmer,[11] Lowy,[22] and others.)

An interesting process and equipment for producing vinegar in an atomizing chamber has been patented recently by Mackin.[23] In this process, the vinegar stock containing vinegar bacteria is sprayed through jet nozzles of a distributor located in the top of a closed chamber. The finely dispersed droplets are allowed to fall through air being turbulently circulated in a countercurrent direction in the chamber. The liquid is collected, cooled to below 95°F, and returned for reatomizing until conversion of the alcohol is complete.

Although not yet of commercial importance, the possibility of oxidizing alcohol to acetic acid by submerged culture of acetic acid bacteria has received some attention. For example, Hromatka and Ebner[17a] compared generator oxidation with submerged-culture oxidation. They found oxygen consumption in generator vinegar production is a linear function of time. In the submerged-culture oxidation, they found that acid formation is an exponential function of time. The submerged-culture process permitted thirty times faster oxidation of the same amount of alcohol. The acetic acid bacteria, during submerged exponential fermentation, had an average $QO_2 = 7{,}750$ ml oxygen per gram dry weight per hour.

## PROCESSING OF VINEGAR

Each finished lot of vinegar is pumped from the generator into storage tanks where it may be allowed to stand for several weeks or months. In some cases, storage is only momentary. In others, the vinegar may stay long enough to age or mature before it is prepared for its final container.

### Aging

Freshly made generator vinegar usually is harsh in flavor and odor when compared with the same vinegar after storing for some time in barrels or tanks. During the period of storage (aging), the harsh flavor and aroma disappear and the vinegar becomes mild, with an agreeable and pleasing flavor and odor. The chemical changes which occur during aging are thought to be similar in many respects to those which occur when wine is stored. Such changes have been discussed in Chapter 7. Esterification is one of

the known changes and may be illustrated by the following equation:

$$\underset{\text{Acetic acid}}{CH_3COOH} + \underset{\text{Ethyl alcohol}}{CH_3CH_2OH} \longrightarrow \underset{\text{Ethyl acetate}}{CH_3COOCH_2CH_3} + H_2O$$

The vinegar should be placed in well-filled wooden barrels or tanks for aging. Unless the vinegar is stored in air-tight containers some of the acetic acid may be oxidized by the vinegar bacteria. *Acetobacter aceti,* found in most generators, and the cellulose-forming *Acetobacter xylinum* are species representative of the "over-oxidizing" vinegar bacteria. These overoxidizing bacteria may be active in the generators and, if supplied with enough air, cause the oxidation of the acetic acid which they have already produced. The complete oxidation of acetic acid caused by these bacteria is represented by the following stoichiometric equation:

$$\underset{\text{Acetic acid}}{CH_3COOH} + \underset{\text{Oxygen}}{O_2} \xrightarrow{\text{Acetobacter enzymes}} \underset{\text{Carbon dioxide}}{2\,CO_2} + \underset{\text{Water}}{2\,H_2O}$$

Care should be taken to use good, sound, clean cooperage for aging. Oak, redwood, cypress, or other wood which does not impart a noticeable off-flavor may be used.

It is not economical or always necessary to store vinegar for aging. Cider and wine vinegars do improve in flavor and aroma when aged. However, only the best-quality stocks of these vinegars destined for use as condiment vinegars are aged in any quantity. Distilled vinegar, which is so commonly used as an ingredient in some other food products, is used generally within a short time after its production and, if stored, is not stored for the specific purpose of aging.

## Clarification

Vinegar must, according to custom, be brilliantly clear when offered for sale. Therefore, it is necessary to clarify it. Clarification usually is effected by some system of filtration, although, under certain conditions, satisfactory clearing may occasionally result from simple settling. Under special circumstances, fining may be desirable.

Any filtration system, regardless of the type (plate and frame, horizontal plate, pad, screen or pulp filters) will be satisfactory for clarification of most vinegars, if operated intelligently.

The use of diatomaceous silica (infusorial earth) will materially increase the rate of effective filtration of vinegar. It is mandatory to use filter aid with plate and frame or screen-type filters. Diatomaceous silica may also be used effectively as an aid with various horizontal-plate filters.

Ordinary fining agents which may be used for the clarification of vinegar include egg albumen, casein, gelatin, isinglass, and bentonite. These clarification agents cause coagulation and settling of the colloidal particles in the cloudy vinegar, either by direct chemical combination with the particles or by neutralizing their electrical charge. After appropriate laboratory tests, the selected fining agent is thoroughly mixed with the vinegar. The vinegar is circulated while the agent is added slowly until finely dispersed throughout. This mixture is then allowed to stand until the suspended matter settles out. If the fining has been successful, the supernatant vinegar is brilliantly clear and can be racked off from the more or less compact sediment.

In practice, however, only a small quantity of vinegar is ever clarified by fining, principally because of the uncertainty of results and the loss of the vinegar left in the sediment after fining. Clarification by filtration is much more certain to be successful and is employed because of its cheapness and because large volumes of vinegar can be handled rapidly. However, corrodable metal should be rigorously avoided at all points of contact with the stock or vinegar. Unless this is done, neither clarification nor fining will give a stable bottled cider or wine vinegar, or any vinegar which can be used with safety in the manufacture of other food products. A special fining procedure should be used for the removal of copper and iron from vinegar.

## Sterilization of Vinegar

After clarification by filtration or fining, the vinegar frequently develops "mother" in the bottom of the container, or a thick membrane at the surface, or even becomes cloudy throughout because the acetic acid bacteria have started to grow again. The growth of these bacteria in the vinegar may be prevented by pasteurization, by chemical sterilization, or by sterile filtration.

Vinegar may be pasteurized in one of three ways: (1) in bulk: the vinegar is then cooled and filled into bottles, jugs, or barrels and sealed; (2) by continuous flash pasteurization: the heated

vinegar is filled directly into bottles or jugs and sealed; (3) by bottle pasteurization; the vinegar is filled into bottles or jugs and pasteurized by immersion in the water bath.

The acetic acid bacteria are not particularly heat resistant, although some of the data found in the literature are somewhat contradictory. Because conditions of general sanitation vary so much in practice, the temperature data as given here have a reasonable safety factor added, which practice has shown to be adequate to handle all degrees of sanitation in the vinegar plants. If the vinegar is to be pasteurized in bulk (in pasteurizing tanks), it is customary to heat it to 140° to 150°F for at least 30 minutes, cool it to about 90° to 100°F, and then fill it into bottles, jugs, or barrels.

For flash pasteurization, the vinegar is made hot enough to provide a center temperature of 150° to 160°F in the bottle at time of closure. If the vinegar is to be bottle pasteurized, the glass containers filled with cold vinegar are immersed in a water bath held at a temperature of 150° to 160°F for sufficient time to bring the center temperature to 150°F. The containers of vinegar are then closed and cooled.

Control of the vinegar bacteria may also be effected by the use of chemical preservatives. However, there are certain legal requirements which have discouraged the general use of chemical preservatives for vinegar, although sulfur dioxide, benzoic acid, and silver ions have been used.

Sterile or germproof filtration may also be used to prevent the growth of acetic acid bacteria in vinegar. This method of sterilization requires a filtration technique which will remove all of the bacteria from the vinegar. It also requires a high level of sanitation which insures that the filtered vinegar will be bottled under aseptic conditions into sterile containers. Sterile or germproof filtration is especially to be recommended for the best wine vinegars whose quality might be impaired by heat. It is to be stressed, however, that the method should not be used unless under the direction of qualified personnel.

### Containers for Vinegar

Although some vinegar is still sold in bulk from barrels, most of it is sold through retail channels in various sizes of glass containers, principally pint and quart bottles and half-gallon and gallon jugs. The glass containers must be well filled and tightly

closed to prevent access of air. Screw-cap closures are commonly used. Some of the large plants employ vapor-vacuum type closures. Regardless of the type, the closure, if metal, must be well protected against the corrosive action of the vinegar.

Vinegar to be used in the food industries may be sold in barrel lots or in truck or railroad tank car quantities. Formerly, most of the bulk vinegar was handled in oak barrels. Now spruce or fir barrels are used extensively. The barrels are heavily coated with paraffin to protect the vinegar from off-flavors or odors coming from the wood. Truck and railroad tank cars may be made of wood, glass-lined metal, resistant stainless steel, or other corrosionproof metal, or plain metal whose interior surfaces have been covered with some impervious coating to prevent contact of the vinegar with the metal.

## DEFECTS OF VINEGAR

### Metallic Contamination

Although the acetic acid bacteria frequently are the chief cause of the deterioration of bottled vinegars, persistent ignorance of the seriousness of metallic contamination probably causes a greater economic loss of vinegar and other food products. It is well known that iron contamination may, if concentrated enough, cause darkening or clouding of vinegar; copper and tin may cause clouding; zinc will produce a poisonous zinc acetate; and all of the metallic ions will, in solution, adversely affect the taste of vinegar. However, in spite of scientific knowledge that metallic contamination of bottled vinegars is undesirable, its full significance is not as widely recognized as its economic importance would merit. This is particularly true in cases where large quantities of copper or iron originally contained in the vinegar may cause deleterious changes in food products prepared with such vinegar. Copper probably is the most serious offender, although in most cases copper and iron are found as simultaneous contaminants of the vinegar.

Vinegar contaminated with copper still is unwittingly used to prepare liquors and brines used in the pickling of cucumbers, green vegetables, green peppers, green olives, capers, and similar pickled foods which contain chlorophyll. The copper from the vinegar replaces the magnesium in the chlorophyll molecule, and an unnatural green pickled product results. Furthermore, the taste

also becomes metallic. Only a few (1 to 5) parts per million of copper are required to change the color of these pickled products perceptibly. The unnatural, artificial, green to blue-green color is already intense with only 10 ppm of copper in the brine. The intensity of the artificial color and metallic off-flavor increases in direct proportion to the copper content.

Undesirable clouding of cider or wine vinegar will occur with comparatively small amounts of copper contamination just as with wines. Cider and wine vinegars contain enough accessory substances to produce the copper complex which causes the turbidity called "copper casse" in the wine industry. Distilled vinegar, however, although it may contain 25 ppm of copper, does not cloud because it does not contain enough accessory substances. Therefore, absence of clouding is no guarantee that distilled vinegar contains no copper.

Although "black vinegar" probably is the most publicized defect caused by iron contamination, the common turbidity of vinegar caused by iron occurs more frequently. Blackening of vinegar is caused by the reaction of iron and tannins contained in the vinegar to form an iron-tannate complex which is black, bluish black, or greenish black. According to Campbell, 100 ppm of iron is required to produce black vinegar. This defect now apparently is of rare occurrence, in the western United States at least, because of the low concentration of tannins found in most vinegars or the original stocks except, of course, those stored in improperly treated new cooperage or those overfined with gelatin and tannins.

Unlike black vinegar, turbid or hazy vinegar resulting from the reaction of iron with phosphates occurs rather frequently because the concentration of phosphates is higher than that of the tannins necessary for the development of black vinegar. Vinegar containing the colloidal iron phosphates complex has a light-colored, slightly bluish, irridescent haze. Such turbidity is sometimes called "blue casse" or "white casse" in the wine industry to distinguish it from the "black casse" or "blue casse" which is caused by the reaction of iron with tannins. The nomenclature of the various types of "casse" obviously has been confused unnecessarily in some of the enological literature. It is suggested, therefore. to use a more specific nomenclature (i.e. ferric or iron phosphates turbidity; ferric or iron tannates blackening; cuprous or copper clouding) and to discontinue the use of the various forms of "casse."

As already mentioned, vinegar contaminated with iron is quite objectionable for use in other food products. The iron contained in such vinegar causes undesirable darkening of brines used to cover pickled cucumbers, olives, and vegetables. It also causes an undesirable graying of pickled vegetables, such as cauliflower, onions, garlic, Japanese white radishes, and horse radish, as well as a discoloration of olives and cucumbers. In addition, the iron causes a noticeable metallic off-flavor in all of the commodities so affected.

Both copper and iron are also known to accelerate rancidification of fats and oils. Therefore, although the writer knows of no supporting experimental data, the copper and iron contained in contaminated vinegar conceivably might accelerate rancidification of fats in food products which contain vinegar as an ingredient.

The copper in solution in vinegar reacts only in the reduced (cuprous) form with the accessory compounds to produce the reversible colloidal copper complex. On oxidation, the colloidal copper complex will disappear. However, with iron also present as a contaminant, complete clearing of the vinegar will never occur because of the simultaneous formation of the iron phosphates complex. In the reduced (ferrous) form, iron will not react with tannins or phosphates. However, once the iron is oxidized to the ferric state, it will produce the defects already described. Nonetheless, a metallic turbidity of vinegar could not be attributed to either copper or iron without confirmatory analyses, because they usually are found as simultaneous contaminants of vinegars.

Vinegar stocks and vinegars become contaminated with copper and iron largely through contact with corrodable metal alloys used in equipment. Metal pick-up occurs in vinegar because of the extremely corrosive action of the acetic acid it contains.

Hose connections, valves, pipe lines, pumps, heat interchangers, spargers, filters, pasteurizers, bottling equipment, tank trucks or cans, funnels, bottle caps, generator packing, etc., may contribute to undesirable metal contamination. Of the generator packings, coke and certain unglazed ceramic materials, such as Raschig rings may contain excessive amounts of iron which must be removed by careful washing with dilute mineral acid, followed by vinegar and then water. To prevent metal contamination, corrosion-resistant metals or other materials should be used at all points where the stock of vinegar contacts equipment surfaces.

Only the most resistant alloys of stainless steel, glass, or other noncorrodable materials, such as hard rubber, pressed paper, and wood should be used in contact with vinegar. Metals, such as aluminum, galvanized iron, or tin, cadmium, nickel and other plate, brass and bronze alloys, and certain stainless-steel alloys, should be avoided. In the first place, they are not resistant enough to the corrosive action of vinegar to be economical to use. Furthermore, some of them, particularly cadmium and zinc, may form poisonous compounds in the vinegar.

Although various stabilization and clarification treatments have been recommended to stabilize vinegar against metal clouding, they do not remove much of the copper or iron. Once the vinegar is contaminated, the only sure way to recover it for general food use is to remove the copper and iron by a special fining procedure in which potassium ferrocyanide is used. Treatment with potassium ferrocyanide, known as "Blauschönung" or "blue fining," requires very careful control and should be done only under the direct supervision of a competent chemist. The use of "blue fining" in the United States is not practiced and vinegar producers prefer to prevent copper and iron contamination by use of the corrosion-resistant materials already mentioned.

## Browning of Vinegar

Enzymatic browning of apple juice, caused by the oxidative enzyme polyphenoloxidase, is well known. Similar, but much less active, enzymatic browning of juices made from several white grape varieties has been described. However, it is conjectural whether oxidative enzyme activity persists in finished vinegar made from either of these two raw materials, especially if the vinegar has been pasteurized. In the light of the newer knowledge of nonenzymatic browning of food products, in general, it seems much more likely that simple browning of bottled vinegar may result from nonenzymatic chemical reactions.[34]

## Bacterial Defects

As already stressed, the acetic acid bacteria, themselves, may cause the deterioration of vinegar. The harmful vinegar bacteria destroy acetic acid. One of them, *Acetobacter xylinum,* is present in almost all vinegar factories where it produces the "mother of vinegar" in cider or wine vinegar. In these finished vinegars, it

forms a slimy sediment at the bottom of the bottle, or a slimy, leathery membrane on the surface, depending on the amount of air available. *Acetobacter aceti* and other species also decompose acetic acid rapidly under favorable conditions and cause turbidity in finished vinegars if air is available. These "overoxidizing" species also cause deterioration of the color of red wine vinegars if allowed to grow in them for any length of time. Control of the bacteria, as already mentioned, is accomplished by elimination of the air supply, pasteurization, use of chemical agents, or by "sterile filtration."

## Vinegar Eels

The vinegar eel, *Anguillula aceti* (Figure 70), is a tiny, aquatic nematode worm frequently found in generators, in vinegar in storage, and in finished vinegar. These tiny organisms multiply rapidly and live in vinegar for a comparatively long time if supplied with sufficient air. The presence of eels in vinegar is unsightly and, although not harmful otherwise, offends man's esthetic senses. Control is possible through adequate sanitation, pasteurization, or filtration of the vinegar and by elimination of air.

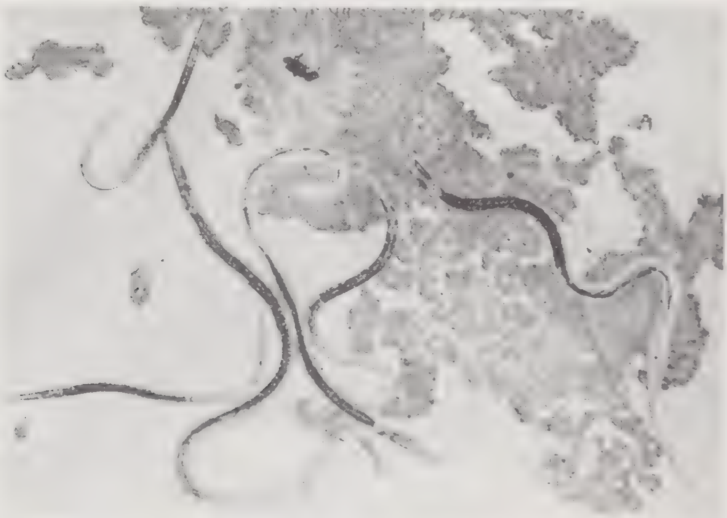


FIGURE 70. *A Colony of Vinegar Eels* (×45)

## Vinegar Mites

Species of mycophagous and predaceous mites or lice (Figure 71) often live in and around the air vents of generators or any

place where the wood is always moist and a supply of food is available. Under favorable conditions of food supply, temperature and moisture, the mites multiply rapidly and may present a problem of sanitation. Control is effected by elimination or prevention of access to feeding places. Light mineral oil spread around the air vents will prevent entry into the generator. The use of steam is suggested to destroy infestations in generators, barrels, etc., since many insecticides may impart undesirable flavors and aromas. Furthermore, some insecticide residues left in food products may be considered to be adulterations by regulatory agencies.

FIGURE 71. *A Predaceous Mite of the Genus* Iphidulus, *Recovered from a Cider Vinegar Generator in Oakland, Calif.* (×70)

### Vinegar Flies

The vinegar fly, *Drosophila melanogaster,* is quite common in vinegar plants and frequently presents a problem in sanitation. The flies can be prevented from becoming a nuisance by keeping the plant clean and tightly screened and by venting the generator stacks to the outside atmosphere. Access of the flies to vinegar or vinegar stock should also be prevented. Judicious use of insecticides is also recommended.

## THE ACETIC ACID BACTERIA

Bacteria of the genus *Acetobacter,* commonly called acetic acid or vinegar bacteria, include the important group of oxidative bacteria whose chief industrial function is the oxidation of ethyl alcohol to produce acetic acid (vinegar). The genus *Acetobacter* Beijerinck[1] has the following characteristics:

Individual cells are ellipsoidal to long and rod-shaped, occurring singly, in pairs, or in short or long chains. Involution forms occur which have spherical, elongated, filamentous, club-shaped, swollen, curved and even branched cells (Figure 72). The young cells are Gram negative. Endospores are not formed. If motile, the cells have polar flagellation.

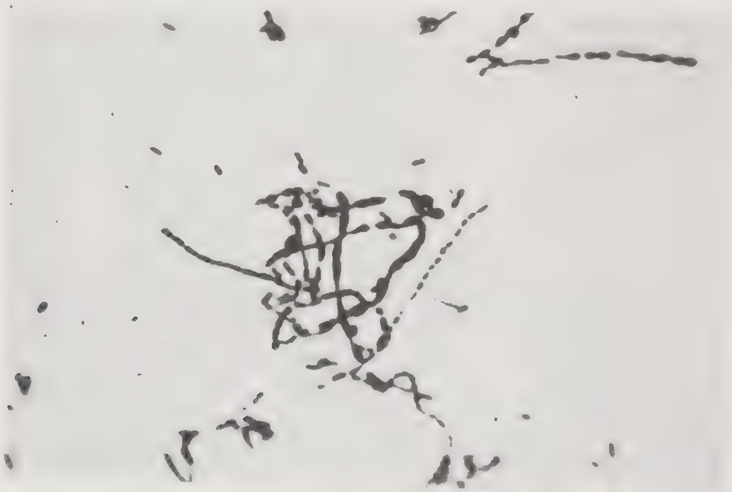


FIGURE 72. *Involution Forms of* Acetobacter aceti

The members of the genus are all obligate aerobes. All species are catalase positive. The genus is chemoheterotrophic, oxidizing various organic compounds to organic acids and other oxidation products which may undergo further oxidation. Common oxidation products are acetic acid from alcohol, gluconic and sometimes keto-gluconic acid from glucose, dihydroxyacetone from glycerol, sorbose from sorbitol, etc.

The nutritional requirements vary from simple to complex. The optimum temperature varies with the species. Members of the genus are widely distributed in nature where they are particularly abundant in plant materials undergoing alcoholic fermentation.

The bacteria are of particular importance to man for their role in the completion of the carbon cycle in nature and for the production of vinegar, certain other organic compounds, and in the deterioration of foods.

The type species is *Acetobacter aceti* (Kützing) Beijerinck.

Henneberg,[16] in his classification of the acetic acid bacteria, has perpetuated the idea that a definite species has a specific habitat; i.e., wort, beer, wine, or generator vinegar (Schnellessig) bacteria. Henneberg lists the type species, *Acetobacter aceti,* with the beer-vinegar bacteria, although this species probably is the most widely distributed in nature of all of the vinegar bacteria. Therefore, it is obvious that this idea is not tenable, as has been pointed out by Vaughn[41] and Shimwell.[32]

If the vinegar bacteria must be differentiated, from the utilitarian point of view, it is much more accurate and just as useful to classify them on the basis of their ability to oxidize a substrate, such as alcohol or glucose. This type of treatment segregates the undesirable species from the desirable ones, regardless of the origin:

(1) Species which cause complete oxidation of the substrate.

(2) Species which cause incomplete oxidation of the substrate.

A key to the species of *Acetobacter* based on this system was proposed by Vaughn[41] and further extended by him[42] in Bergey's *Manual of Determinative Bacteriology.* The key was developed and extended on the basis of earlier work by Visser't Hooft.[44] The system is workable and reasonably accurate, as is shown by the acceptance by Shimwell.[32] This classification has been developed on the basis of broad taxonomic lines. It does not include the many technological names which are abundant in some of the literature on vinegar.

All bacterial taxonomy and nomenclature is based on priority and is governed by a rigid code. The industrial bacteriologist or chemist, who uses bacteria to produce specific chemicals or foods, should pay the same attention to the differentiation and nomenclature of the bacteria as he does to the differentiation and nomenclature of the compounds produced by their activity. Unless this obligation is accepted, the present confusion can only increase.

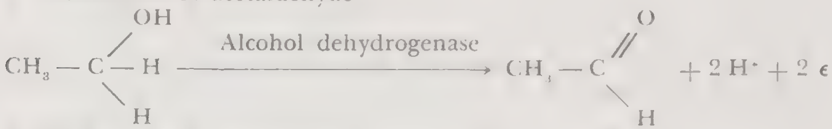
## MECHANISM OF ACETIC ACID FORMATION

The oxidation of alcohol to acetic acid is the result of dehydrogenation reactions, involving the cytochrome system. Acetalde-

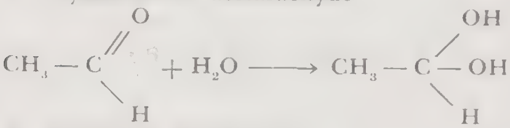
hyde, the principal intermediate, was first detected by Hoyer.[17]

The conversion of alcohol to acetic acid may be represented by the following reactions:

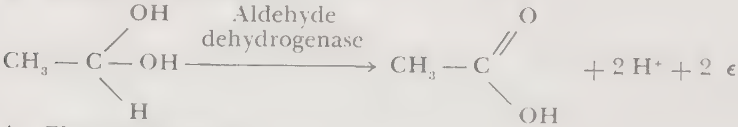
1. Formation of acetaldehyde



2. Hydration of acetaldehyde



3. Formation of acetic acid



4. Electron transfer

$$4\ H^+ + 4\ \epsilon + O_2 \xrightarrow{\text{Cytochrome system}} 2\ H_2O$$

It should be stressed that these reactions represent stoichiometric acetification. What effect the amounts of alcohol, the air supply, and different acetic acid bacteria may have on over-all acetification is, in many cases, conjectural. For further reading, the publications of Bernhauer,[2] Butlin,[6] Oppenheimer and Stern,[26] Michaelis,[24] Porter,[29] and Prescott and Dunn[30] are suggested.

## COMPETING PROCESSES

Edible acetic acid or vinegar must be of biological origin. Therefore, there is no competition in this field. Suggestions have been made to use fermentation as a source of industrial acetic acid. Microbiological acetic acid might, of course, be concentrated, probably best by vapor-phase solvent-extraction procedures, such as are used in modern wood-distillation plants for recovering acetic acid from pyroligneous acid. However, the costs of concentration make this process uneconomic for the production of industrial acetic acid in competition with the synthetic production of this commodity from acetylene or through catalytic oxidation of ethanol.

## BIBLIOGRAPHY

1a. Allgeier, R. J., R. T. Wisthoff, and F. M. Hildebrandt, *Ind. Eng. Chem.*, **44**, 669 (1952).

1b. Allgeier, R. J., R. T. Wisthoff, and F. M. Hildebrandt, *Ind. Eng. Chem.*, **45**, 489 (1953).
1. Beijerinck, M. W., *Proc. Kon Akad. Wetenschapp., Amsterdam*, **2**, 495 (1900).
2. Bernhauer, K., *Die oxydativen Gärungen*, Berlin, Springer, 1932.
3. Bioletti, F. T., "Grape Vinegar," *Calif. Agr. Expt. Sta. Bull. 227* (1912).
4. Bioletti, F. T., *Microbiology*, Chapter IV, p. 636-648, Philadelphia, Blakiston, 1921.
5. Boerhaave, H., *Elementa Chemiae. Lugduni Batavorum*, **2**, 179-207 (1732). (Original not seen; cited by Mitchell, Pasteur.)
6. Butlin, K. R., "Survey of the Biochemical Activity of the Acetic Acid Bacteria," Gr. Br. Dept. Sci. Ind. Research, Chem. Research, *Special Report No. 2*, London, H. M. Stationery Office, 1936.
7. Campbell, C. H., *Campbell's Book—Canning, Preserving and Pickling*, Rev. ed., Chicago, Vance Publishing Corp., 1937.
8. Chatfield, Charlotte, and Laura I. McLaughlin, "Proximate Composition of Fresh Fruits," *U. S. Dept. of Agr. Circ. 50* (1931).
9. Cruess, W. V., "Vinegar from Waste Fruits," *Calif. Agr. Exp. Sta. Bull. 287* (1917).
10. Cruess, W. V., *Commercial Fruit and Vegetable Products*, 3rd. ed., New York, McGraw-Hill, 1948.
11. Elmer, L. S., U. S. Patent 2,156,428 (1939).
12. Fabian, F. W., "Honey Vinegar," *Mich. State Coll. Ext. Bull. 149* (1935).
13. Fetzer, W. R., *Food Industries*, **2**, 489 (1930).
14. Frings, H., U. S. Patent 1,880,381 (1932).
15. Hansen, A. E., *Food Industries*, **7**, 277 (1935).
16. Henneberg, W., *Handbuch der Gärungsbakteriologie*, vols. I and II, 2nd. ed., Berlin, Paul Parey, 1926.
16a. Hildebrandt, F. M., *Food Inds.*, **13**, No. 8, 47 (1941).
17. Hoyer, D. P., *Bijdrage tot de kennis van de azijnbacteriën*, Dissertation, Univ. Leiden, Delft, Waltman, 1898.
17a. Hromatka, O., and H. Ebner, *Enzymologia*, **13**, 369 (1949).
18. Hydraulic Press Mfg. Co., *Vinegar Hand Book*, Mt. Gilead, Ohio, Hydraulic Press Mfg. Co., 1919.
19. Joslyn, M. A., and W. V. Cruess, "Home and Farm Preparation of Vinegar," *Calif. Agr. Exp. Sta. Circular 332* (1934).
20. Kützing, F., *J. prakt. Chem.*, **11**, 385 (1837).

21. Lavoisier, A. L., *Traité élémentaire de chimie.,* 2nd ed., **1**, 159 (1793). (Original not seen; cited by Pasteur.)
22. Lowy, J., *Food Industries,* **13**, 47 (1941).
23. Mackin, J. J., U. S. Patent 2,423,897 (1947).
24. Michaelis, L., *Fundamentals of Oxidation and Reduction, Currents in Biochemical Research,* p. 207, New York, Interscience, 1946.
25. Mitchell, C. A., *Vinegar: Its Manufacture and Examination,* 2nd. ed., London, Griffin, 1926.
26. Oppenheimer, C., and K. G. Stern, *Biological Oxidation,* New York, Nordemann Publishing Co., 1939.
27. Pasteur, L., *Études sur le vinaigre,* Paris, Masson and Sons, 1868.
28. Persoon, C. H., *Mycologia Europaea,* Section 1, 96 (1822).
29. Porter, J. R., *Bacterial Chemistry and Physiology,* New York, John Wiley, 1946.
30. Prescott, S. C., and C. G. Dunn, *Industrial Microbiology,* 2nd. ed., New York, McGraw-Hill, 1949.
31. Rozier, F., *Dictionnaire universel d'agriculture,* **4**, 525 (1786). (Original not seen; cited by Pasteur.)
32. Shimwell, J. L., *Wallerstein Labs. Commun.,* **32**, 27 (1948).
33. Speas, V. E., U. S. Patent 2,153,849 (1939).
34. Stadtman, E. R., *Advances in Food Research,* **1**, 325 (1948).
35. Tolman, L. M., *J. Assoc. Off. Agr. Chem.,* **1939**, 30 (1939).
36. U. S. Dept. of Agr. Service and Regulatory Announcements, *Food and Drug No. 2,* Fifth Revision, Washington, D. C., U. S. Govt. Printing Office, November 1936.
37. U. S. Treasury Dept., Bureau of Internal Revenue, *Regulations 3,* "Industrial Alcohol," Washington, D. C., U. S. Govt. Printing Office, 1942.
38. U. S. Treasury Dept., Bureau of Internal Revenue, *Appendix to Regulations 3,* "Formulae for Completely and Specially Denatured Alcohol," Washington, D. C., U. S. Govt. Printing Office, 1942.
39. U. S. Treasury Dept., Bureau of Internal Revenue, *Regulations 7,* "Wine," Washington, D. C., U. S. Govt. Printing Office, 1945.
40. U. S. Treasury Dept., Bureau of Internal Revenue, *Regulations 19,* "Production of Vinegar by the Vaporizing Process," Washington, D. C., U. S. Govt. Printing Office, 1940.
41. Vaughn, R. H., *Wallerstein Labs. Commun.,* **5**, 5 (1942).
42. Vaughn, R. H., in *Bergey's Manual of Determinative Bacteriology,* 6th ed., Baltimore, Williams and Wilkins, 1948.

43. Verona, O., *Microbiologia delle fermentazioni e microbiologia industriale,* Firenze, Luigi Macri, 1950.
44. Visser't Hooft, F., *Biochemische onderzoekingen over het geslacht Acetobacter,* Dissertation, Tech. Hoogeschool, Delft, 1925.
45. Wüstenfeld, H., *Lehrbuch der Essigfabrikation,* Berlin, Paul Parey, 1930.

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